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(71) Applicants (for all designated States except US): CHIRON CORPORATION [US/US]; 4560 Horton Street, Emeryville, CA 94608 (US). THE INSTITUTE FOR GENOMIC RESEARCH [US/US]; 9212 Medical Center Drive, Rockville, MD 20850 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): PIZZA, Mariagrazia [IT/IT]; Chiron SpA, Via Fiorentina, 1, I-53100 Siena (IT). HICKEY, Erin [US/US]; 4569 Horton Street, Emeryville, CA 94608-2916 (US). PETERSON, Jeremy [US/US]; 4569 Horton Street, Emeryville, CA 94608-2916 (US). TETTELIN, Herve [US/US]; 4569 Horton Street, Emeryville, CA 94608-2916 (US). VENTER, J., Craig [US/US]; 4569 Horton Street, Emeryville, CA 94608-2916 (US). MASIGNANI, Vega [IT/IT]; Chiron SpA, Via Fiorentina, 1, I-53100 Siena (IT). GALEOTTI, Cesira

[IT/IT]; Chiron SpA, Via Fiorentina 1, I-53100 Siena (IT). MORA, Marirosa [IT/IT]; Chiron SpA, Via Fiorentina, 1, I-53100 Siena (IT). RATTI, Giulio [IT/IT]; Chiron SpA, Via Fiorentina, 1, I-53100 Siena (IT). SCARSELLI, Maria [IT/IT]; Chiron SpA, Via Fiorentina, 1, I-53100 Siena (IT). SCARLATO, Vincenzo [IT/IT]; Chiron SpA, Via Fiorentina 1, I-53100 Siena (IT). RAPPUOLI, Rino [IT/IT]; Chiron SpA, Via Fiorentina 1, I-53100 Siena (IT). FRAZER, Claire, M. [US/US]; 4560 Horton Street, Emeryville, CA 94608 (US). GRANDI, Guido [IT/IT]; Chiron SpA, Via Fiorentina, 1, I-53100 Siena (IT).

(74) Agent: HARBIN, Alisa, A.; Chiron Corporation, Intellectual Property - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US).

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(54) Title: NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE

A

919 (48 kDa)

PURIFICATION

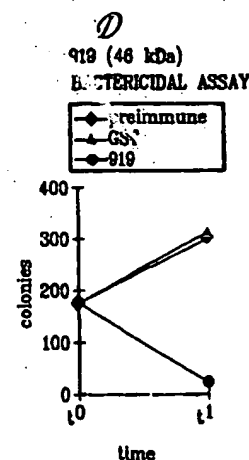
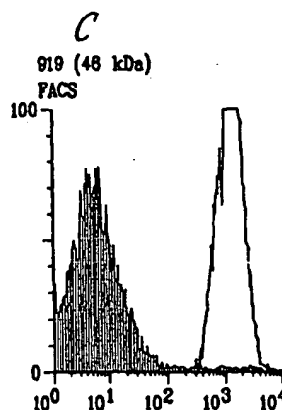
MI 919

B

919 (48 kDa)

WESTERN BLOT

OMV TP PP



E

919 (48 kDa)

ELISA assay: positive

(57) Abstract

The invention provides methods of obtaining immunogenic proteins from genomic sequences including *Neisseria*, including the amino acid sequences and the corresponding nucleotide sequences, as well as the genomic sequence of *Neisseria meningitidis B*. The proteins so obtained are useful antigens for vaccines, immunogenic compositions, and/or diagnostics.

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NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE

This application claims priority to provisional U.S. application serial no. 60/132,068, filed 30 April 1999; PCT/US99/23573, filed 8 October 1999 (to be published April 2000); and Great Britain application serial no. GB-0004695.3, filed 28 February 2000.

This invention relates to methods of obtaining antigens and immunogens, the antigens and immunogens so obtained, and nucleic acids from the bacterial species: *Neisseria meningitidis*. In particular, it relates to genomic sequences from the bacterium; more particularly its "B" serogroup.

BACKGROUND

Neisseria meningitidis is a non-motile, gram negative diplococcus human pathogen. It colonizes the pharynx, causing meningitis and, occasionally, septicaemia in the absence of meningitis. It is closely related to *N. gonorrhoea*, although one feature that clearly differentiates meningococcus from gonococcus is the presence of a polysaccharide capsule that is present in all pathogenic meningococci.

N. meningitidis causes both endemic and epidemic disease. In the United States the attack rate is 0.6-1 per 100,000 persons per year, and it can be much greater during outbreaks. (see Lieberman *et al.* (1996) Safety and Immunogenicity of a Serogroups A/C *Neisseria meningitidis* Oligosaccharide-Protein Conjugate Vaccine in Young Children. *JAMA* 275(19):1499-1503; Schuchat *et al* (1997) Bacterial Meningitis in the United States in 1995. *N Engl J Med* 337(14):970-976). In developing countries, endemic disease rates are much higher and during epidemics incidence rates can reach 500 cases per 100,000 persons per year. Mortality is extremely high, at 10-20% in the United States, and much higher in developing countries. Following the introduction of the conjugate vaccine against *Haemophilus influenzae*, *N. meningitidis* is the major cause of bacterial meningitis at all ages in the United States (Schuchat *et al* (1997) *supra*).

Based on the organism's capsular polysaccharide, 12 serogroups of *N. meningitidis* have been identified. Group A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the

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United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the United States and developed countries. The meningococcal vaccine currently in use is a tetravalent polysaccharide vaccine composed of serogroups A, C, Y and W135. Although efficacious in adolescents and adults, it induces a poor immune response and short duration of protection, and cannot be used in infants (e.g., Morbidity and Mortality weekly report, Vol. 46, No. RR-5 (1997)). This is because polysaccharides are T-cell independent antigens that induce a weak immune response that cannot be boosted by repeated immunization. Following the success of the vaccination against *H. influenzae*, conjugate vaccines against serogroups A and C have been developed and are at the final stage of clinical testing (Zollinger WD "New and Improved Vaccines Against Meningococcal Disease". In: *New Generation Vaccines, supra*, pp. 469-488; Lieberman *et al* (1996) *supra*; Costantino *et al* (1992) Development and phase I clinical testing of a conjugate vaccine against meningococcus A (menA) and C (menC) (*Vaccine* 10:691-698)).

Meningococcus B (MenB) remains a problem, however. This serotype currently is responsible for approximately 50% of total meningitis in the United States, Europe, and South America. The polysaccharide approach cannot be used because the MenB capsular polysaccharide is a polymer of $\alpha(2-8)$ -linked *N*-acetyl neuraminic acid that is also present in mammalian tissue. This results in tolerance to the antigen; indeed, if an immune response were elicited, it would be anti-self, and therefore undesirable. In order to avoid induction of autoimmunity and to induce a protective immune response, the capsular polysaccharide has, for instance, been chemically modified substituting the *N*-acetyl groups with *N*-propionyl groups, leaving the specific antigenicity unaltered (Romero & Outschoorn (1994) Current status of Meningococcal group B vaccine candidates: capsular or non-capsular? *Clin Microbiol Rev* 7(4):559-575).

Alternative approaches to MenB vaccines have used complex mixtures of outer membrane proteins (OMPs), containing either the OMPs alone, or OMPs enriched in porins, or deleted of the class 4 OMPs that are believed to induce antibodies that block bactericidal activity. This approach produces vaccines that are not well characterized. They are able to protect against the homologous strain, but are not effective at large where there are many antigenic variants of the outer membrane proteins. To overcome the antigenic variability, multivalent vaccines containing up to nine different porins have been constructed (e.g.,

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Poolman JT (1992) Development of a meningococcal vaccine. *Infect. Agents Dis.* 4:13-28). Additional proteins to be used in outer membrane vaccines have been the opa and opc proteins, but none of these approaches have been able to overcome the antigenic variability (e.g., Ala'Aldeen & Borriello (1996) The meningococcal transferrin-binding proteins 1 and 2 are both surface exposed and generate bactericidal antibodies capable of killing homologous and heterologous strains. *Vaccine* 14(1):49-53).

A certain amount of sequence data is available for meningococcal and gonococcal genes and proteins (e.g., EP-A-0467714, WO96/29412), but this is by no means complete. The provision of further sequences could provide an opportunity to identify secreted or surface-exposed proteins that are presumed targets for the immune system and which are not antigenically variable or at least are more antigenically conserved than other and more variable regions. Thus, those antigenic sequences that are more highly conserved are preferred sequences. Those sequences specific to *Neisseria meningitidis* or *Neisseria gonorrhoeae* that are more highly conserved are further preferred sequences. For instance, some of the identified proteins could be components of efficacious vaccines against meningococcus B, some could be components of vaccines against all meningococcal serotypes, and others could be components of vaccines against all pathogenic *Neisseriae*. The identification of sequences from the bacterium will also facilitate the production of biological probes, particularly organism-specific probes.

It is thus an object of the invention is to provide *Neisserial* DNA sequences which (1) encode proteins predicted and/or shown to be antigenic or immunogenic, (2) can be used as probes or amplification primers, and (3) can be analyzed by bioinformatics.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the products of protein expression and purification of the predicted ORF 919 as cloned and expressed in *E. coli*.

Fig. 2 illustrates the products of protein expression and purification of the predicted ORF 279 as cloned and expressed in *E. coli*.

Fig. 3 illustrates the products of protein expression and purification of the predicted ORF 576-1 as cloned and expressed in *E. coli*.

Fig. 4 illustrates the products of protein expression and purification of the predicted ORF 519-1 as cloned and expressed in *E. coli*.

Fig. 5 illustrates the products of protein expression and purification of the predicted ORF 121-1 as cloned and expressed in *E. coli*.

Fig. 6 illustrates the products of protein expression and purification of the predicted ORF 128-1 as cloned and expressed in *E. coli*.

Fig. 7 illustrates the products of protein expression and purification of the predicted ORF 206 as cloned and expressed in *E. coli*.

Fig. 8 illustrates the products of protein expression and purification of the predicted ORF 287 as cloned and expressed in *E. coli*.

Fig. 9 illustrates the products of protein expression and purification of the predicted ORF 406 as cloned and expressed in *E. coli*.

Fig. 10 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 919 as cloned and expressed in *E. coli*.

Fig. 11 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 279 as cloned and expressed in *E. coli*.

Fig. 12 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 576-1 as cloned and expressed in *E. coli*.

Fig. 13 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 519-1 as cloned and expressed in *E. coli*.

Fig. 14 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 121-1 as cloned and expressed in *E. coli*.

Fig. 15 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 128-1 as cloned and expressed in *E. coli*.

Fig. 16 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 206 as cloned and expressed in *E. coli*.

Fig. 17 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 287 as cloned and expressed in *E. coli*.

Fig. 18 illustrates the hydrophilicity plot, antigenic index and AMPHI regions of the products of protein expression the predicted ORF 406 as cloned and expressed in *E. coli*.

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THE INVENTION

The first complete sequence of the genome of *N. meningitidis* was disclosed as 961 partial contiguous nucleotide sequences, shown as SEQ ID NOs:1-961 of co-owned PCT/US99/23573 (the '573 application), filed 8 October 1999 (to be published April 2000). A single sequence full length genome of *N. meningitidis* was also disclosed as SEQ ID NO. 1068 of the '573 application. The invention is based on a full length genome of *N. meningitidis* which appears as SEQ ID NO. 1 in the present application as Appendix A hereto. The 961 sequences of the '573 application represent substantially the whole genome of serotype B of *N. meningitidis* (>99.98%). There is partial overlap between some of the 961 contiguous sequences ("contigs") shown in the 961 sequences, which overlap was used to construct the single full length sequence shown in SEQ ID NO. 1 in Appendix A hereto, using the TIGR Assembler [G.S. Sutton et al., *TIGR Assembler: A New Tool for Assembling Large Shotgun Sequencing Projects*, Genome Science and Technology, 1:9-19 (1995)]. Some of the nucleotides in the contigs had been previously released. (See ftp://ftp.tigr.org/pub/data/n_meningitidis on the world-wide web or "WWW"). The coordinates of the 2508 released sequences in the present contigs are presented in Appendix A of the '573 application. These data include the contig number (or i.d.) as presented in the first column; the name of the sequence as found on WWW is in the second column; with the coordinates of the contigs in the third and fourth columns, respectively. The sequences of certain MenB ORFs presented in Appendix B of the '573 application feature in International Patent Application filed by Chiron SpA on October 9, 1998 (PCT/IB98/01665) and January 14, 1999 (PCT/IB99/00103) respectively. Appendix B hereto provides a listing of 2158 open reading frames contained within the full length sequence found in SEQ ID NO. 1 in Appendix A hereto. The information set forth in Appendix B hereto includes the "NMB" name of the sequence, the putative translation product, and the beginning and ending nucleotide positions within SEQ ID NO. 1 which comprise the open reading frames. These open reading frames are referred to herein as the "NMB open reading frames".

In a first aspect, the invention provides nucleic acid including the *N. meningitidis* nucleotide sequence shown in SEQ ID NO. 1 in Appendix A hereto. It also provides nucleic acid comprising sequences having sequence identity to the nucleotide sequence disclosed herein. Depending on the particular sequence, the degree of sequence identity is preferably

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greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more). These sequences include, for instance, mutants and allelic variants. The degree of sequence identity cited herein is determined across the length of the sequence determined by the Smith-Waterman homology search algorithm as implemented in MPSRCH program (Oxford Molecular) using an affine gap search with the following parameters: gap open penalty 12, gap extension penalty 1.

The invention also provides nucleic acid including a fragment of one or more of the nucleotide sequences set out herein, including the NMB open reading frames shown in Appendix B hereto. The fragment should comprise at least n consecutive nucleotides from the sequences and, depending on the particular sequence, n is 10 or more (e.g., 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 60, 75, 100 or more). Preferably, the fragment is unique to the genome of *N. meningitidis*, that is to say it is not present in the genome of another organism. More preferably, the fragment is unique to the genome of strain B of *N. meningitidis*. The invention also provides nucleic acid that hybridizes to those provided herein. Conditions for hybridizing are disclosed herein.

The invention also provides nucleic acid including sequences complementary to those described above (e.g., for antisense, for probes, or for amplification primers).

Nucleic acid according to the invention can, of course, be prepared in many ways (e.g., by chemical synthesis, from DNA libraries, from the organism itself, etc.) and can take various forms (e.g., single-stranded, double-stranded, vectors, probes, primers, etc.). The term "nucleic acid" includes DNA and RNA, and also their analogs, such as those containing modified backbones, and also peptide nucleic acid (PNA) etc.

It will be appreciated that, as SEQ ID NOs:1-961 of the '573 application represent the substantially complete genome of the organism, with partial overlap, references to SEQ ID NOs:1-961 of the '573 application include within their scope references to the complete genomic sequence, that is, SEQ ID NO. 1 hereof. For example, where two SEQ ID NOs overlap, the invention encompasses the single sequence which is formed by assembling the two overlapping sequences, which full sequence will be found in SEQ ID NO. 1 hereof. Thus, for instance, a nucleotide sequence which bridges two SEQ ID NOs but is not present in its entirety in either SEQ ID NO is still within the scope of the invention. Such a sequence will be present in its entirety in the single full length sequence of SEQ ID NO. 1 of the present application.

The invention also provides vectors including nucleotide sequences of the invention (e.g., expression vectors, sequencing vectors, cloning vectors, etc.) and host cells transformed with such vectors.

According to a further aspect, the invention provides a protein including an amino acid sequence encoded within a *N. meningitidis* nucleotide sequence set out herein. It also provides proteins comprising sequences having sequence identity to those proteins. Depending on the particular sequence, the degree of sequence identity is preferably greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more). Sequence identity is determined as above disclosed. These homologous proteins include mutants and allelic variants, encoded within the *N. meningitidis* nucleotide sequence set out herein.

The invention further provides proteins including fragments of an amino acid sequence encoded within a *N. meningitidis* nucleotide sequence set out in the sequence listing. The fragments should comprise at least n consecutive amino acids from the sequences and, depending on the particular sequence, n is 7 or more (e.g., 8, 10, 12, 14, 16, 18, 20 or more). Preferably the fragments comprise an epitope from the sequence.

The proteins of the invention can, of course, be prepared by various means (e.g., recombinant expression, purification from cell culture, chemical synthesis, etc.) and in various forms (e.g. native, fusions etc.). They are preferably prepared in substantially isolated form (i.e., substantially free from other *N. meningitidis* host cell proteins).

Various tests can be used to assess the *in vivo* immunogenicity of the proteins of the invention. For example, the proteins can be expressed recombinantly or chemically synthesized and used to screen patient sera by immunoblot. A positive reaction between the protein and patient serum indicates that the patient has previously mounted an immune response to the protein in question; i.e., the protein is an immunogen. This method can also be used to identify immunodominant proteins.

The invention also provides nucleic acid encoding a protein of the invention.

In a further aspect, the invention provides a computer, a computer memory, a computer storage medium (e.g., floppy disk, fixed disk, CD-ROM, etc.), and/or a computer database containing the nucleotide sequence of nucleic acid according to the invention. Preferably, it contains one or more of the *N. meningitidis* nucleotide sequences set out herein.

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This may be used in the analysis of the *N. meningitidis* nucleotide sequences set out herein. For instance, it may be used in a search to identify open reading frames (ORFs) or coding sequences within the sequences.

In a further aspect, the invention provides a method for identifying an amino acid sequence, comprising the step of searching for putative open reading frames or protein-coding sequences within a *N. meningitidis* nucleotide sequence set out herein. Similarly, the invention provides the use of a *N. meningitidis* nucleotide sequence set out herein in a search for putative open reading frames or protein-coding sequences.

Open-reading frame or protein-coding sequence analysis is generally performed on a computer using standard bioinformatic techniques. Typical algorithms or program used in the analysis include ORFFINDER (NCBI), GENMARK [Borodovsky & McIninch (1993) *Computers Chem* 17:122-133], and GLIMMER [Salzberg et al. (1998) *Nucl Acids Res* 26:544-548].

A search for an open reading frame or protein-coding sequence may comprise the steps of searching a *N. meningitidis* nucleotide sequence set out herein for an initiation codon and searching the upstream sequence for an in-frame termination codon. The intervening codons represent a putative protein-coding sequence. Typically, all six possible reading frames of a sequence will be searched.

An amino acid sequence identified in this way can be expressed using any suitable system to give a protein. This protein can be used to raise antibodies which recognize epitopes within the identified amino acid sequence. These antibodies can be used to screen *N. meningitidis* to detect the presence of a protein comprising the identified amino acid sequence.

Furthermore, once an ORF or protein-coding sequence is identified, the sequence can be compared with sequence databases. Sequence analysis tools can be found at NCBI (<http://www.ncbi.nlm.nih.gov>) e.g., the algorithms BLAST, BLAST2, BLASTn, BLASTp, tBLASTn, BLASTx, & tBLASTx [see also Altschul *et al.* (1997) Gapped BLAST and PSI-BLAST: new generation of protein database search programs. *Nucleic Acids Research* 25:2289-3402]. Suitable databases for comparison include the nonredundant GenBank, EMBL, DDBJ and PDB sequences, and the nonredundant GenBank CDS translations, PDB,

SwissProt, Spupdate and PIR sequences. This comparison may give an indication of the function of a protein.

Hydrophobic domains in an amino acid sequence can be predicted using algorithms such as those based on the statistical studies of Esposti *et al.* [Critical evaluation of the hydropathy of membrane proteins (1990) *Eur J Biochem* 190:207-219]. Hydrophobic domains represent potential transmembrane regions or hydrophobic leader sequences, which suggest that the proteins may be secreted or be surface-located. These properties are typically representative of good immunogens.

Similarly, transmembrane domains or leader sequences can be predicted using the PSORT algorithm (<http://www.psort.nibb.ac.jp>), and functional domains can be predicted using the MOTIFS program (GCG Wisconsin & PROSITE).

The invention also provides nucleic acid including an open reading frame or protein-coding sequence present in a *N. meningitidis* nucleotide sequence set out herein. Furthermore, the invention provides a protein including the amino acid sequence encoded by this open reading frame or protein-coding sequence.

According to a further aspect, the invention provides antibodies which bind to these proteins. These may be polyclonal or monoclonal and may be produced by any suitable means known to those skilled in the art.

The antibodies of the invention can be used in a variety of ways, e.g., for confirmation that a protein is expressed, or to confirm where a protein is expressed. Labeled antibody (e.g., fluorescent labeling for FACS) can be incubated with intact bacteria and the presence of label on the bacterial surface confirms the location of the protein, for instance.

According to a further aspect, the invention provides compositions including protein, antibody, and/or nucleic acid according to the invention. These compositions may be suitable as vaccines, as immunogenic compositions, or as diagnostic reagents.

The invention also provides nucleic acid, protein, or antibody according to the invention for use as medicaments (e.g., as vaccines) or as diagnostic reagents. It also provides the use of nucleic acid, protein, or antibody according to the invention in the manufacture of (i) a medicament for treating or preventing infection due to Neisserial bacteria (ii) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria. Said Neisserial bacteria may be any species or

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strain (such as *N. gonorrhoeae*) but are preferably *N. meningitidis*, especially strain A, strain B or strain C.

In still yet another aspect, the present invention provides for compositions including proteins, nucleic acid molecules, or antibodies. More preferable aspects of the present invention are drawn to immunogenic compositions of proteins. Further preferable aspects of the present invention contemplate pharmaceutical immunogenic compositions of proteins or vaccines and the use thereof in the manufacture of a medicament for the treatment or prevention of infection due to Neisserial bacteria, preferably infection of MenB.

The invention also provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of nucleic acid, protein, and/or antibody according to the invention.

According to further aspects, the invention provides various processes.

A process for producing proteins of the invention is provided, comprising the step of culturing a host cell according to the invention under conditions which induce protein expression. A process which may further include chemical synthesis of proteins and/or chemical synthesis (at least in part) of nucleotides.

A process for detecting polynucleotides of the invention is provided, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridizing conditions to form duplexes; and (b) detecting said duplexes.

A process for detecting proteins of the invention is provided, comprising the steps of: (a) contacting an antibody according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

Another aspect of the present invention provides for a process for detecting antibodies that selectably bind to antigens or polypeptides or proteins specific to any species or strain of Neisserial bacteria and preferably to strains of *N. gonorrhoeae* but more preferably to strains of *N. meningitidis*, especially strain A, strain B or strain C, more preferably MenB, where the process comprises the steps of: (a) contacting antigen or polypeptide or protein according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Methodology - Summary of standard procedures and techniques.

General

This invention provides *Neisseria meningitidis* MenB nucleotide sequences, amino acid sequences encoded therein. With these disclosed sequences, nucleic acid probe assays and expression cassettes and vectors can be produced. The proteins can also be chemically synthesized. The expression vectors can be transformed into host cells to produce proteins. The purified or isolated polypeptides can be used to produce antibodies to detect MenB proteins. Also, the host cells or extracts can be utilized for biological assays to isolate agonists or antagonists. In addition, with these sequences one can search to identify open reading frames and identify amino acid sequences. The proteins may also be used in immunogenic compositions and as vaccine components.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature e.g., Sambrook *Molecular Cloning; A Laboratory Manual, Second Edition* (1989); *DNA Cloning, Volumes I and ii* (D.N Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed, 1984); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds. 1984); *Transcription and Translation* (B.D. Hames & S.J. Higgins eds. 1984); *Animal Cell Culture* (R.I. Freshney ed. 1986); *Immobilized Cells and Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide to Molecular Cloning* (1984); the *Methods in Enzymology* series (Academic Press, Inc.), especially volumes 154 & 155; *Gene Transfer Vectors for Mammalian Cells* (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Mayer and Walker, eds. (1987), *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Scopes, (1987) *Protein Purification: Principles and Practice*, Second Edition (Springer-Verlag, N.Y.), and *Handbook of Experimental Immunology, Volumes I-IV* (D.M. Weir and C.C. Blackwell eds 1986).

Standard abbreviations for nucleotides and amino acids are used in this specification.

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All publications, patents, and patent applications cited herein are incorporated in full by reference.

Expression systems

The *Neisseria* MenB nucleotide sequences can be expressed in a variety of different expression systems; for example those used with mammalian cells, plant cells, baculoviruses, bacteria, and yeast.

i. Mammalian Systems

Mammalian expression systems are known in the art. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g., structural gene) into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, usually located 25-30 base pairs (bp) upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element, usually located within 100 to 200 bp upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation (Sambrook et al. (1989) "Expression of Cloned Genes in Mammalian Cells." In *Molecular Cloning: A Laboratory Manual*, 2nd ed.).

Mammalian viral genes are often highly expressed and have a broad host range; therefore sequences encoding mammalian viral genes provide particularly useful promoter sequences. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), and herpes simplex virus promoter. In addition, sequences derived from non-viral genes, such as the murine metallothionein gene, also provide useful promoter sequences. Expression may be either constitutive or regulated (inducible). Depending on the promoter selected, many promoters may be inducible using known substrates, such as the use of the mouse mammary tumor virus (MMTV) promoter with the glucocorticoid responsive element (GRE) that is induced by glucocorticoid in hormone-responsive transformed cells (see for example, U.S. Patent 5,783,681).

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The presence of an enhancer element (enhancer), combined with the promoter elements described above, will usually increase expression levels. An enhancer is a regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to homologous or heterologous promoters, with synthesis beginning at the normal RNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription initiation site, in either normal or flipped orientation, or at a distance of more than 1000 nucleotides from the promoter (Maniatis et al. (1987) *Science* 236:1237; Alberts et al. (1989) *Molecular Biology of the Cell*, 2nd ed.). Enhancer elements derived from viruses may be particularly useful, because they usually have a broader host range. Examples include the SV40 early gene enhancer (Dijkema et al (1985) *EMBO J.* 4:761) and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus (Gorman et al. (1982b) *Proc. Natl. Acad. Sci.* 79:6777) and from human cytomegalovirus (Boshart et al. (1985) *Cell* 41:521). Additionally, some enhancers are regulatable and become active only in the presence of an inducer, such as a hormone or metal ion (Sassone-Corsi and Borelli (1986) *Trends Genet.* 2:215; Maniatis et al. (1987) *Science* 236:1237).

A DNA molecule may be expressed intracellularly in mammalian cells. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in mammalian cells. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The adenovirus tripartite leader is an example of a leader sequence that provides for secretion of a foreign protein in mammalian cells.

Usually, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the

mature mRNA is formed by site-specific post-transcriptional cleavage and polyadenylation (Birnstiel et al. (1985) *Cell* 41:349; Proudfoot and Whitelaw (1988) "Termination and 3' end processing of eukaryotic RNA. In *Transcription and splicing* (ed. B.D. Hames and D.M. Glover); Proudfoot (1989) *Trends Biochem. Sci.* 14:105). These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator/polyadenylation signals include those derived from SV40 (Sambrook et al (1989) "Expression of cloned genes in cultured mammalian cells." In *Molecular Cloning: A Laboratory Manual*).

Usually, the above-described components, comprising a promoter, polyadenylation signal, and transcription termination sequence are put together into expression constructs. Enhancers, introns with functional splice donor and acceptor sites, and leader sequences may also be included in an expression construct, if desired. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as mammalian cells or bacteria. Mammalian replication systems include those derived from animal viruses, which require trans-acting factors to replicate. For example, plasmids containing the replication systems of papovaviruses, such as SV40 (Gluzman (1981) *Cell* 23:175) or polyomavirus, replicate to extremely high copy number in the presence of the appropriate viral T antigen. Additional examples of mammalian replicons include those derived from bovine papillomavirus and Epstein-Barr virus. Additionally, the replicon may have two replication systems, thus allowing it to be maintained, for example, in mammalian cells for expression and in a prokaryotic host for cloning and amplification. Examples of such mammalian-bacteria shuttle vectors include pMT2 (Kaufman et al. (1989) *Mol. Cell. Biol.* 9:946) and pHEBO (Shimizu et al. (1986) *Mol. Cell. Biol.* 6:1074).

The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection

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(ATCC), including but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines.

ii. Plant Cellular Expression Systems

There are many plant cell culture and whole plant genetic expression systems known in the art. Exemplary plant cellular genetic expression systems include those described in patents, such as: U.S. 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, *Phytochemistry* 30:3861-3863 (1991). Descriptions of plant protein signal peptides may be found in addition to the references described above in Vaulcombe et al., *Mol. Gen. Genet.* 209:33-40 (1987); Chandler et al., *Plant Molecular Biology* 3:407-418 (1984); Rogers, *J. Biol. Chem.* 260:3731-3738 (1985); Rothstein et al., *Gene* 55:353-356 (1987); Whittier et al., *Nucleic Acids Research* 15:2515-2535 (1987); Wirsal et al., *Molecular Microbiology* 3:3-14 (1989); Yu et al., *Gene* 122:247-253 (1992). A description of the regulation of plant gene expression by the phytohormone, gibberellic acid and secreted enzymes induced by gibberellic acid can be found in R.L. Jones and J. MacMillin, Gibberellins: in: *Advanced Plant Physiology*, Malcolm B. Wilkins, ed., 1984 Pitman Publishing Limited, London, pp. 21-52. References that describe other metabolically-regulated genes: Sheen, *Plant Cell*, 2:1027-1038(1990); Maas et al., *EMBO J.* 9:3447-3452 (1990); Benkel and Hickey, *Proc. Natl. Acad. Sci.* 84:1337-1339 (1987)

Typically, using techniques known in the art, a desired polynucleotide sequence is inserted into an expression cassette comprising genetic regulatory elements designed for operation in plants. The expression cassette is inserted into a desired expression vector with companion sequences upstream and downstream from the expression cassette suitable for expression in a plant host. The companion sequences will be of plasmid or viral origin and provide necessary characteristics to the vector to permit the vectors to move DNA from an original cloning host, such as bacteria, to the desired plant host. The basic bacterial/plant vector construct will preferably provide a broad host range prokaryote replication origin; a prokaryote selectable marker; and, for *Agrobacterium* transformations, T DNA sequences for *Agrobacterium*-mediated transfer to plant chromosomes. Where the heterologous gene is not

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readily amenable to detection, the construct will preferably also have a selectable marker gene suitable for determining if a plant cell has been transformed. A general review of suitable markers, for example for the members of the grass family, is found in Wilnink and Dons, 1993, *Plant Mol. Biol. Repr.*, 11(2):165-185.

Sequences suitable for permitting integration of the heterologous sequence into the plant genome are also recommended. These might include transposon sequences and the like for homologous recombination as well as Ti sequences which permit random insertion of a heterologous expression cassette into a plant genome. Suitable prokaryote selectable markers include resistance toward antibiotics such as ampicillin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art.

The nucleic acid molecules of the subject invention may be included into an expression cassette for expression of the protein(s) of interest. Usually, there will be only one expression cassette, although two or more are feasible. The recombinant expression cassette will contain in addition to the heterologous protein encoding sequence the following elements, a promoter region, plant 5' untranslated sequences, initiation codon depending upon whether or not the structural gene comes equipped with one, and a transcription and translation termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the cassette allow for easy insertion into a pre-existing vector.

A heterologous coding sequence may be for any protein relating to the present invention. The sequence encoding the protein of interest will encode a signal peptide which allows processing and translocation of the protein, as appropriate, and will usually lack any sequence which might result in the binding of the desired protein of the invention to a membrane. Since, for the most part, the transcriptional initiation region will be for a gene which is expressed and translocated during germination, by employing the signal peptide which provides for translocation, one may also provide for translocation of the protein of interest. In this way, the protein(s) of interest will be translocated from the cells in which they are expressed and may be efficiently harvested. Typically secretion in seeds are across the aleurone or scutellar epithelium layer into the endosperm of the seed. While it is not required that the protein be secreted from the cells in which the protein is produced, this facilitates the isolation and purification of the recombinant protein.

Since the ultimate expression of the desired gene product will be in a eucaryotic cell it is desirable to determine whether any portion of the cloned gene contains sequences which will be processed out as introns by the host's splicosome machinery. If so, site-directed mutagenesis of the "intron" region may be conducted to prevent losing a portion of the genetic message as a false intron code, Reed and Maniatis, *Cell* 41:95-105, 1985.

The vector can be microinjected directly into plant cells by use of micropipettes to mechanically transfer the recombinant DNA. Crossway, *Mol. Gen. Genet.*, 202:179-185, 1985. The genetic material may also be transferred into the plant cell by using polyethylene glycol, Krens, et al., *Nature*, 296, 72-74, 1982. Another method of introduction of nucleic acid segments is high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface, Klein, et al., *Nature*, 327, 70-73, 1987 and Knudsen and Muller, 1991, *Planta*, 185:330-336 teaching particle bombardment of barley endosperm to create transgenic barley. Yet another method of introduction would be fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies, Fraley, et al., *Proc. Natl. Acad. Sci. USA*, 79, 1859-1863, 1982.

The vector may also be introduced into the plant cells by electroporation. (Fromm et al., *Proc. Natl Acad. Sci. USA* 82:5824, 1985). In this technique, plant protoplasts are electroporated in the presence of plasmids containing the gene construct. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and form plant callus.

All plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be transformed by the present invention so that whole plants are recovered which contain the transferred gene. It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugarcane, sugar beet, cotton, fruit and other trees, legumes and vegetables. Some suitable plants include, for example, species from the genera *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersion*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hererocallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*,

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Ranunculus, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Lolium*, *Zea*, *Triticum*, *Sorghum*, and *Datura*.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts containing copies of the heterologous gene is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced from the protoplast suspension. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Shoots and roots normally develop simultaneously. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is fully reproducible and repeatable.

In some plant cell culture systems, the desired protein of the invention may be excreted or alternatively, the protein may be extracted from the whole plant. Where the desired protein of the invention is secreted into the medium, it may be collected. Alternatively, the embryos and embryoless-half seeds or other plant tissue may be mechanically disrupted to release any secreted protein between cells and tissues. The mixture may be suspended in a buffer solution to retrieve soluble proteins. Conventional protein isolation and purification methods will be then used to purify the recombinant protein. Parameters of time, temperature pH, oxygen, and volumes will be adjusted through routine methods to optimize expression and recovery of heterologous protein.

iii. Baculovirus Systems

The polynucleotide encoding the protein can also be inserted into a suitable insect expression vector, and is operably linked to the control elements within that vector. Vector construction employs techniques which are known in the art. Generally, the components of the expression system include a transfer vector, usually a bacterial plasmid, which contains both a fragment of the baculovirus genome, and a convenient restriction site for insertion of the heterologous gene or genes to be expressed; a wild type baculovirus with a sequence homologous to the baculovirus-specific fragment in the transfer vector (this allows for the

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homologous recombination of the heterologous gene in to the baculovirus genome); and appropriate insect host cells and growth media.

After inserting the DNA sequence encoding the protein into the transfer vector, the vector and the wild type viral genome are transfected into an insect host cell where the vector and viral genome are allowed to recombine. The packaged recombinant virus is expressed and recombinant plaques are identified and purified. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA ("MaxBac" kit). These techniques are generally known to those skilled in the art and fully described in Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987) (hereinafter "Summers and Smith").

Prior to inserting the DNA sequence encoding the protein into the baculovirus genome, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are usually assembled into an intermediate transplacement construct (transfer vector). This construct may contain a single gene and operably linked regulatory elements; multiple genes, each with its owned set of operably linked regulatory elements; or multiple genes, regulated by the same set of regulatory elements. Intermediate transplacement constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as a bacterium. The replicon will have a replication system, thus allowing it to be maintained in a suitable host for cloning and amplification.

Currently, the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. Many other vectors, known to those of skill in the art, have also been designed. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 basepairs downstream from the ATT; see Luckow and Summers, *Virology* (1989) 17:31.

The plasmid usually also contains the polyhedrin polyadenylation signal (Miller et al. (1988) *Ann. Rev. Microbiol.*, 42:177) and a prokaryotic ampicillin-resistance (*amp*) gene and origin of replication for selection and propagation in *E. coli*.

Baculovirus transfer vectors usually contain a baculovirus promoter. A baculovirus promoter is any DNA sequence capable of binding a baculovirus RNA polymerase and initiating the downstream (5' to 3') transcription of a coding sequence (e.g., structural gene)

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into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A baculovirus transfer vector may also have a second domain called an enhancer, which, if present, is usually distal to the structural gene. Expression may be either regulated or constitutive.

Structural genes, abundantly transcribed at late times in a viral infection cycle, provide particularly useful promoter sequences. Examples include sequences derived from the gene encoding the viral polyhedron protein, Friesen et al., (1986) "The Regulation of Baculovirus Gene Expression," in: *The Molecular Biology of Baculoviruses* (ed. Walter Doerfler); EPO Publ. Nos. 127 839 and 155 476; and the gene encoding the p10 protein, Vlcek et al., (1988), *J. Gen. Virol.* 69:765.

DNA encoding suitable signal sequences can be derived from genes for secreted insect or baculovirus proteins, such as the baculovirus polyhedrin gene (Carbonell et al. (1988) *Gene*, 73:409). Alternatively, since the signals for mammalian cell posttranslational modifications (such as signal peptide cleavage, proteolytic cleavage, and phosphorylation) appear to be recognized by insect cells, and the signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells, leaders of non-insect origin, such as those derived from genes encoding human (alpha) α -interferon, Maeda et al., (1985), *Nature* 315:592; human gastrin-releasing peptide, Lebacqz-Verheyden et al., (1988), *Molec. Cell. Biol.* 8:3129; human IL-2, Smith et al., (1985) *Proc. Nat'l Acad. Sci. USA*, 82:8404; mouse IL-3, (Miyajima et al., (1987) *Gene* 58:273; and human glucocerebrosidase, Martin et al. (1988) *DNA*, 7:99, can also be used to provide for secretion in insects.

A recombinant polypeptide or polyprotein may be expressed intracellularly or, if it is expressed with the proper regulatory sequences, it can be secreted. Good intracellular expression of nonfused foreign proteins usually requires heterologous genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal. If desired, methionine at the N-terminus may be cleaved from the mature protein by *in vitro* incubation with cyanogen bromide.

Alternatively, recombinant polyproteins or proteins which are not naturally secreted can be secreted from the insect cell by creating chimeric DNA molecules that encode a fusion

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protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in insects. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the translocation of the protein into the endoplasmic reticulum.

After insertion of the DNA sequence and/or the gene encoding the expression product precursor of the protein, an insect cell host is co-transformed with the heterologous DNA of the transfer vector and the genomic DNA of wild type baculovirus -- usually by co-transfection. The promoter and transcription termination sequence of the construct will usually comprise a 2-5kb section of the baculovirus genome. Methods for introducing heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summers and Smith *supra*; Ju et al. (1987); Smith et al., *Mol. Cell. Biol.* (1983) 3:2156; and Luckow and Summers (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous double crossover recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. Miller et al., (1989), *Bioessays* 4:91. The DNA sequence, when cloned in place of the polyhedrin gene in the expression vector, is flanked both 5' and 3' by polyhedrin-specific sequences and is positioned downstream of the polyhedrin promoter.

The newly formed baculovirus expression vector is subsequently packaged into an infectious recombinant baculovirus. Homologous recombination occurs at low frequency (between about 1% and about 5%); thus, the majority of the virus produced after cotransfection is still wild-type virus. Therefore, a method is necessary to identify recombinant viruses. An advantage of the expression system is a visual screen allowing recombinant viruses to be distinguished. The polyhedrin protein, which is produced by the native virus, is produced at very high levels in the nuclei of infected cells at late times after viral infection. Accumulated polyhedrin protein forms occlusion bodies that also contain embedded particles. These occlusion bodies, up to 15 μm in size, are highly refractile, giving them a bright shiny appearance that is readily visualized under the light microscope. Cells infected with recombinant viruses lack occlusion bodies. To distinguish recombinant virus from wild-type virus, the transfection supernatant is plaqued onto a monolayer of insect cells by techniques known to those skilled in the art. Namely, the plaques are screened under the light microscope for the presence (indicative of wild-type virus) or absence (indicative of

recombinant virus) of occlusion bodies. *Current Protocols in Microbiology* Vol. 2 (Ausubel et al. eds) at 16.8 (Supp. 10, 1990); Summers and Smith, *supra*; Miller et al. (1989).

Recombinant baculovirus expression vectors have been developed for infection into several insect cells. For example, recombinant baculoviruses have been developed for, *inter alia*: *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni* (PCT Pub. No. WO 89/046699; Carbonell et al., (1985) *J. Virol.* 56:153; Wright (1986) *Nature* 321:718; Smith et al., (1983) *Mol. Cell. Biol.* 3:2156; and see generally, Fraser, et al. (1989) *In Vitro Cell. Dev. Biol.* 25:225).

Cells and cell culture media are commercially available for both direct and fusion expression of heterologous polypeptides in a baculovirus/expression system; cell culture technology is generally known to those skilled in the art. See, e.g., Summers and Smith *supra*.

The modified insect cells may then be grown in an appropriate nutrient medium, which allows for stable maintenance of the plasmid(s) present in the modified insect host. Where the expression product gene is under inducible control, the host may be grown to high density, and expression induced. Alternatively, where expression is constitutive, the product will be continuously expressed into the medium and the nutrient medium must be continuously circulated, while removing the product of interest and augmenting depleted nutrients. The product may be purified by such techniques as chromatography, e.g., HPLC, affinity chromatography, ion exchange chromatography, etc.; electrophoresis; density gradient centrifugation; solvent extraction, or the like. As appropriate, the product may be further purified, as required, so as to remove substantially any insect proteins which are also secreted in the medium or result from lysis of insect cells, so as to provide a product which is at least substantially free of host debris, e.g., proteins, lipids and polysaccharides.

In order to obtain protein expression, recombinant host cells derived from the transformants are incubated under conditions which allow expression of the recombinant protein encoding sequence. These conditions will vary, dependent upon the host cell selected. However, the conditions are readily ascertainable to those of ordinary skill in the art, based upon what is known in the art.

iv. Bacterial Systems

Bacterial expression techniques are known in the art. A bacterial promoter is any DNA sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A bacterial promoter may also have a second domain called an operator, that may overlap an adjacent RNA polymerase binding site at which RNA synthesis begins. The operator permits negative regulated (inducible) transcription, as a gene repressor protein may bind the operator and thereby inhibit transcription of a specific gene. Constitutive expression may occur in the absence of negative regulatory elements, such as the operator. In addition, positive regulation may be achieved by a gene activator protein binding sequence, which, if present is usually proximal (5') to the RNA polymerase binding sequence. An example of a gene activator protein is the catabolite activator protein (CAP), which helps initiate transcription of the lac operon in *Escherichia coli* (*E. coli*) (Raibaud *et al.* (1984) *Annu. Rev. Genet.* 18:173). Regulated expression may therefore be either positive or negative, thereby either enhancing or reducing transcription.

Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) (Chang *et al.* (1977) *Nature* 198:1056), and maltose. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (*trp*) (Goeddel *et al.* (1980) *Nuc. Acids Res.* 8:4057; Yelverton *et al.* (1981) *Nucl. Acids Res.* 9:731; U.S. Patent 4,738,921; EPO Publ. Nos. 036 776 and 121 775). The beta-lactamase (*bla*) promoter system (Weissmann (1981) "The cloning of interferon and other mistakes." In *Interferon 3* (ed. I. Gresser)), bacteriophage lambda PL (Shimatake *et al.* (1981) *Nature* 292:128) and T5 (U.S. Patent 4,689,406) promoter systems also provide useful promoter sequences.

In addition, synthetic promoters which do not occur in nature also function as bacterial promoters. For example, transcription activation sequences of one bacterial or bacteriophage promoter may be joined with the operon sequences of another bacterial or bacteriophage promoter, creating a synthetic hybrid promoter (U.S. Patent 4,551,433). For

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example, the *tac* promoter is a hybrid *trp-lac* promoter comprised of both *trp* promoter and *lac* operon sequences that is regulated by the *lac* repressor (Amann *et al.* (1983) *Gene* 25:167; de Boer *et al.* (1983) *Proc. Natl. Acad. Sci.* 80:21). Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. A naturally occurring promoter of non-bacterial origin can also be coupled with a compatible RNA polymerase to produce high levels of expression of some genes in prokaryotes. The bacteriophage T7 RNA polymerase/promoter system is an example of a coupled promoter system (Studier *et al.* (1986) *J. Mol. Biol.* 189:113; Tabor *et al.* (1985) *Proc Natl. Acad. Sci.* 82:1074). In addition, a hybrid promoter can also be comprised of a bacteriophage promoter and an *E. coli* operator region (EPO Publ. No. 267 851).

In addition to a functioning promoter sequence, an efficient ribosome binding site is also useful for the expression of foreign genes in prokaryotes. In *E. coli*, the ribosome binding site is called the Shine-Dalgarno (SD) sequence and includes an initiation codon (ATG) and a sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon (Shine *et al.* (1975) *Nature* 254:34). The SD sequence is thought to promote binding of mRNA to the ribosome by the pairing of bases between the SD sequence and the 3' end of *E. coli* 16S rRNA (Steitz *et al.* (1979) "Genetic signals and nucleotide sequences in messenger RNA." In *Biological Regulation and Development: Gene Expression* (ed. R.F. Goldberg)). To express eukaryotic genes and prokaryotic genes with weak ribosome-binding site, it is often necessary to optimize the distance between the SD sequence and the ATG of the eukaryotic gene (Sambrook *et al.* (1989) "Expression of cloned genes in *Escherichia coli*." In *Molecular Cloning: A Laboratory Manual*).

A DNA molecule may be expressed intracellularly. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide or by either *in vivo* or *in vitro* incubation with a bacterial methionine N-terminal peptidase (EPO Publ. No. 219 237).

Fusion proteins provide an alternative to direct expression. Usually, a DNA sequence encoding the N-terminal portion of an endogenous bacterial protein, or other stable protein, is

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fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the bacteriophage lambda cell gene can be linked at the 5' terminus of a foreign gene and expressed in bacteria. The resulting fusion protein preferably retains a site for a processing enzyme (factor Xa) to cleave the bacteriophage protein from the foreign gene (Nagai *et al.* (1984) *Nature* 309:810). Fusion proteins can also be made with sequences from the *lacZ* (Jia *et al.* (1987) *Gene* 60:197), *trpE* (Allen *et al.* (1987) *J. Biotechnol.* 5:93; Makoff *et al.* (1989) *J. Gen. Microbiol.* 135:11), and *Chey* (EPO Publ. No. 324 647) genes. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (e.g. ubiquitin specific processing-protease) to cleave the ubiquitin from the foreign protein. Through this method, native foreign protein can be isolated (Miller *et al.* (1989) *Bio/Technology* 7:698).

Alternatively, foreign proteins can also be secreted from the cell by creating chimeric DNA molecules that encode a fusion protein comprised of a signal peptide sequence fragment that provides for secretion of the foreign protein in bacteria (U.S. Patent 4,336,336). The signal sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). Preferably there are processing sites, which can be cleaved either *in vivo* or *in vitro* encoded between the signal peptide fragment and the foreign gene.

DNA encoding suitable signal sequences can be derived from genes for secreted bacterial proteins, such as the *E. coli* outer membrane protein gene (*ompA*) (Masui *et al.* (1983), in: *Experimental Manipulation of Gene Expression*; Ghayeb *et al.* (1984) *EMBO J.* 3:2437) and the *E. coli* alkaline phosphatase signal sequence (*phoA*) (Oka *et al.* (1985) *Proc. Natl. Acad. Sci.* 82:7212). As an additional example, the signal sequence of the alpha-amylase gene from various *Bacillus* strains can be used to secrete heterologous proteins from *B. subtilis* (Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. No. 244 042).

Usually, transcription termination sequences recognized by bacteria are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the

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coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Transcription termination sequences frequently include DNA sequences of about 50 nucleotides capable of forming stem loop structures that aid in terminating transcription. Examples include transcription termination sequences derived from genes with strong promoters, such as the *trp* gene in *E. coli* as well as other biosynthetic genes.

Usually, the above described components, comprising a promoter, signal sequence (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as bacteria. The replicon will have a replication system, thus allowing it to be maintained in a prokaryotic host either for expression or for cloning and amplification. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably contain at least about 10, and more preferably at least about 20 plasmids. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host.

Alternatively, the expression constructs can be integrated into the bacterial genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various *Bacillus* strains integrate into the *Bacillus* chromosome (EPO Publ. No. 127 328). Integrating vectors may also be comprised of bacteriophage or transposon sequences.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of bacterial strains that have been transformed. Selectable markers can be expressed in the bacterial host and may include genes which render bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin (neomycin), and tetracycline (Davies *et al.* (1978) *Annu. Rev. Microbiol.* 32:469). Selectable

markers may also include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways.

Alternatively, some of the above described components can be put together in transformation vectors. Transformation vectors are usually comprised of a selectable marker that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extra-chromosomal replicons or integrating vectors, have been developed for transformation into many bacteria. For example, expression vectors have been developed for, *inter alia*, the following bacteria: *Bacillus subtilis* (Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. Nos. 036 259 and 063 953; PCT Publ. No. WO 84/04541), *Escherichia coli* (Shimatake *et al.* (1981) *Nature* 292:128; Amann *et al.* (1985) *Gene* 40:183; Studier *et al.* (1986) *J. Mol. Biol.* 189:113; EPO Publ. Nos. 036 776, 136 829 and 136 907), *Streptococcus cremoris* (Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655); *Streptococcus lividans* (Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655), *Streptomyces lividans* (U.S. Patent 4,745,056).

Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and usually include either the transformation of bacteria treated with CaCl_2 or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation. Transformation procedures usually vary with the bacterial species to be transformed. (See e.g., use of *Bacillus*: Masson *et al.* (1989) *FEMS Microbiol. Lett.* 60:273; Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EPO Publ. Nos. 036 259 and 063 953; PCT Publ. No. WO 84/04541; use of *Campylobacter*: Miller *et al.* (1988) *Proc. Natl. Acad. Sci.* 85:856; and Wang *et al.* (1990) *J. Bacteriol.* 172:949; use of *Escherichia coli*: Cohen *et al.* (1973) *Proc. Natl. Acad. Sci.* 69:2110; Dower *et al.* (1988) *Nucleic Acids Res.* 16:6127; Kushner (1978) "An improved method for transformation of *Escherichia coli* with ColE1-derived plasmids. In *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering* (eds. H.W. Boyer and S. Nicosia); Mandel *et al.* (1970) *J. Mol. Biol.* 53:159; Taketo (1988) *Biochim. Biophys. Acta* 949:318; use of *Lactobacillus*: Chassy *et al.* (1987) *FEMS Microbiol. Lett.* 44:173; use of *Pseudomonas*: Fiedler *et al.* (1988) *Anal. Biochem.* 170:38; use of *Staphylococcus*: Augustin *et al.* (1990) *FEMS Microbiol. Lett.* 66:203; use of *Streptococcus*: Barany *et al.* (1980) *J. Bacteriol.* 144:698;

Harlander (1987) "Transformation of *Streptococcus lactis* by electroporation, in: *Streptococcal Genetics* (ed. J. Ferretti and R. Curtiss III); Perry *et al.* (1981) *Infect. Immun.* 32:1295; Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655; Somkuti *et al.* (1987) *Proc. 4th Evr. Cong. Biotechnology* 1:412.

v. Yeast Expression

Yeast expression systems are also known to one of ordinary skill in the art. A yeast promoter is any DNA sequence capable of binding yeast RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site (the "TATA Box") and a transcription initiation site. A yeast promoter may also have a second domain called an upstream activator sequence (UAS), which, if present, is usually distal to the structural gene. The UAS permits regulated (inducible) expression. Constitutive expression occurs in the absence of a UAS. Regulated expression may be either positive or negative, thereby either enhancing or reducing transcription.

Yeast is a fermenting organism with an active metabolic pathway, therefore sequences encoding enzymes in the metabolic pathway provide particularly useful promoter sequences. Examples include alcohol dehydrogenase (ADH) (EPO Publ. No. 284 044), enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase (GAP or GAPDH), hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, and pyruvate kinase (PyK) (EPO Publ. No. 329 203). The yeast *PHO5* gene, encoding acid phosphatase, also provides useful promoter sequences (Myanohara *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:1).

In addition, synthetic promoters which do not occur in nature also function as yeast promoters. For example, UAS sequences of one yeast promoter may be joined with the transcription activation region of another yeast promoter, creating a synthetic hybrid promoter. Examples of such hybrid promoters include the ADH regulatory sequence linked to the GAP transcription activation region (U.S. Patent Nos. 4,876,197 and 4,880,734). Other examples of hybrid promoters include promoters which consist of the regulatory sequences of

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either the *ADH2*, *GAL4*, *GAL10*, OR *PHO5* genes, combined with the transcriptional activation region of a glycolytic enzyme gene such as GAP or PyK (EPO Publ. No. 164 556). Furthermore, a yeast promoter can include naturally occurring promoters of non-yeast origin that have the ability to bind yeast RNA polymerase and initiate transcription. Examples of such promoters include, *inter alia*, (Cohen *et al.* (1980) *Proc. Natl. Acad. Sci. USA* 77:1078; Henikoff *et al.* (1981) *Nature* 283:835; Hollenberg *et al.* (1981) *Curr. Topics Microbiol. Immunol.* 96:119; Hollenberg *et al.* (1979) "The Expression of Bacterial Antibiotic Resistance Genes in the Yeast *Saccharomyces cerevisiae*," in: *Plasmids of Medical, Environmental and Commercial Importance* (eds. K.N. Timmis and A. Puhler); Mercerau-Puigalon *et al.* (1980) *Gene* 11:163; Panthier *et al.* (1980) *Curr. Genet.* 2:109;).

A DNA molecule may be expressed intracellularly in yeast. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Fusion proteins provide an alternative for yeast expression systems, as well as in mammalian, plant, baculovirus, and bacterial expression systems. Usually, a DNA sequence encoding the N-terminal portion of an endogenous yeast protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the yeast or human superoxide dismutase (SOD) gene, can be linked at the 5' terminus of a foreign gene and expressed in yeast. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. See e.g., EPO Publ. No. 196056. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (e.g. ubiquitin-specific processing protease) to cleave the ubiquitin from the foreign protein. Through this method, therefore, native foreign protein can be isolated (e.g., WO88/024066).

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provide for secretion in yeast of the foreign protein. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can

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be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell.

DNA encoding suitable signal sequences can be derived from genes for secreted yeast proteins, such as the yeast invertase gene (EPO Publ. No. 012 873; JPO Publ. No. 62:096,086) and the A-factor gene (U.S. Patent 4,588,684). Alternatively, leaders of non-yeast origin, such as an interferon leader, exist that also provide for secretion in yeast (EPO Publ. No. 060 057).

A preferred class of secretion leaders are those that employ a fragment of the yeast alpha-factor gene, which contains both a "pre" signal sequence, and a "pro" region. The types of alpha-factor fragments that can be employed include the full-length pre-pro alpha factor leader (about 83 amino acid residues) as well as truncated alpha-factor leaders (usually about 25 to about 50 amino acid residues) (U.S. Patent Nos. 4,546,083 and 4,870,008; EPO Publ. No. 324 274). Additional leaders employing an alpha-factor leader fragment that provides for secretion include hybrid alpha-factor leaders made with a presequence of a first yeast, but a pro-region from a second yeast alpha factor. (See e.g., PCT Publ. No. WO 89/02463.)

Usually, transcription termination sequences recognized by yeast are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator sequence and other yeast-recognized termination sequences, such as those coding for glycolytic enzymes.

Usually, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as yeast or bacteria. The replicon may have two replication systems, thus allowing it to be maintained, for example, in yeast for expression and in a prokaryotic host for cloning and amplification. Examples of such yeast-bacteria shuttle vectors include YEp24 (Botstein *et al.* (1979) *Gene* 8:17-24), pCI/1 (Brake *et al.* (1984) *Proc. Natl. Acad. Sci USA* 81:4642-4646), and YRp17 (Stinchcomb *et al.* (1982) *J. Mol. Biol.* 158:157). In addition, a replicon may be

either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably have at least about 10, and more preferably at least about 20. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host. See e.g., Brake *et al.*, *supra*.

Alternatively, the expression constructs can be integrated into the yeast genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to a yeast chromosome that allows the vector to integrate, and preferably contain two homologous sequences flanking the expression construct. Integrations appear to result from recombinations between homologous DNA in the vector and the yeast chromosome (Orr-Weaver *et al.* (1983) *Methods in Enzymol.* 101:228-245). An integrating vector may be directed to a specific locus in yeast by selecting the appropriate homologous sequence for inclusion in the vector. See Orr-Weaver *et al.*, *supra*. One or more expression construct may integrate, possibly affecting levels of recombinant protein produced (Rine *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:6750). The chromosomal sequences included in the vector can occur either as a single segment in the vector, which results in the integration of the entire vector, or two segments homologous to adjacent segments in the chromosome and flanking the expression construct in the vector, which can result in the stable integration of only the expression construct.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of yeast strains that have been transformed. Selectable markers may include biosynthetic genes that can be expressed in the yeast host, such as *ADE2*, *HIS4*, *LEU2*, *TRP1*, and *ALG7*, and the G418 resistance gene, which confer resistance in yeast cells to tunicamycin and G418, respectively. In addition, a suitable selectable marker may also provide yeast with the ability to grow in the presence of toxic compounds, such as metal. For example, the presence of *CUP1* allows yeast to grow in the presence of copper ions (Butt *et al.* (1987) *Microbiol. Rev.* 51:351).

Alternatively, some of the above described components can be put together into transformation vectors. Transformation vectors are usually comprised of a selectable marker

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that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extrachromosomal replicons or integrating vectors, have been developed for transformation into many yeasts. For example, expression vectors and methods of introducing exogenous DNA into yeast hosts have been developed for, *inter alia*, the following yeasts: *Candida albicans* (Kurtz, *et al.* (1986) *Mol. Cell. Biol.* 6:142); *Candida maltosa* (Kunze, *et al.* (1985) *J. Basic Microbiol.* 25:141); *Hansenula polymorpha* (Gleeson, *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302); *Kluyveromyces fragilis* (Das, *et al.* (1984) *J. Bacteriol.* 158:1165); *Kluyveromyces lactis* (De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:737; Van den Berg *et al.* (1990) *Bio/Technology* 8:135); *Pichia guilliermondii* (Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141); *Pichia pastoris* (Cregg, *et al.* (1985) *Mol. Cell. Biol.* 5:3376; U.S. Patent Nos. 4,837,148 and 4,929,555); *Saccharomyces cerevisiae* (Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163); *Schizosaccharomyces pombe* (Beach and Nurse (1981) *Nature* 300:706); and *Yarrowia lipolytica* (Davidow, *et al.* (1985) *Curr. Genet.* 10:380471 Gaillardin, *et al.* (1985) *Curr. Genet.* 10:49).

Methods of introducing exogenous DNA into yeast hosts are well-known in the art, and usually include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Transformation procedures usually vary with the yeast species to be transformed. See e.g., [Kurtz *et al.* (1986) *Mol. Cell. Biol.* 6:142; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; *Candida*]; [Gleeson *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302; *Hansenula*]; [Das *et al.* (1984) *J. Bacteriol.* 158:1165; De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:1165; Van den Berg *et al.* (1990) *Bio/Technology* 8:135; *Kluyveromyces*]; [Cregg *et al.* (1985) *Mol. Cell. Biol.* 5:3376; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; U.S. Patent Nos. 4,837,148 and 4,929,555; *Pichia*]; [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163 *Saccharomyces*]; [Beach and Nurse (1981) *Nature* 300:706; *Schizosaccharomyces*]; [Davidow *et al.* (1985) *Curr. Genet.* 10:39; Gaillardin *et al.* (1985) *Curr. Genet.* 10:49; *Yarrowia*].

Definitions

A composition containing X is "substantially free of" Y when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95% or even 99% by weight.

The term "heterologous" refers to two biological components that are not found together in nature. The components may be host cells, genes, or regulatory regions, such as promoters. Although the heterologous components are not found together in nature, they can function together, as when a promoter heterologous to a gene is operably linked to the gene. Another example is where a Neisserial sequence is heterologous to a mouse host cell.

An "origin of replication" is a polynucleotide sequence that initiates and regulates replication of polynucleotides, such as an expression vector. The origin of replication behaves as an autonomous unit of polynucleotide replication within a cell, capable of replication under its own control. An origin of replication may be needed for a vector to replicate in a particular host cell. With certain origins of replication, an expression vector can be reproduced at a high copy number in the presence of the appropriate proteins within the cell. Examples of origins are the autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

A "mutant" sequence is defined as a DNA, RNA or amino acid sequence differing from but having homology with the native or disclosed sequence. Depending on the particular sequence, the degree of homology between the native or disclosed sequence and the mutant sequence is preferably greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, 99% or more) which is calculated as described above. As used herein, an "allelic variant" of a nucleic acid molecule, or region, for which nucleic acid sequence is provided herein is a nucleic acid molecule, or region, that occurs at essentially the same locus in the genome of another or second isolate, and that, due to natural variation caused by, for example, mutation or recombination, has a similar but not identical nucleic acid sequence. A coding region allelic variant typically encodes a protein having similar activity to that of the protein encoded by the gene to which it is being compared. An allelic variant can also comprise an alteration in the 5' or 3' untranslated regions of the gene, such as in regulatory control regions. (see, for example, U.S. Patent 5,753,235).

Antibodies

As used herein, the term "antibody" refers to a polypeptide or group of polypeptides composed of at least one antibody combining site. An "antibody combining site" is the three-dimensional binding space with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows a binding of the antibody with the antigen. "Antibody" includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, humanized antibodies, altered antibodies, univalent antibodies, Fab proteins, and single domain antibodies.

Antibodies against the proteins of the invention are useful for affinity chromatography, immunoassays, and distinguishing/identifying *Neisseria* MenB proteins. Antibodies elicited against the proteins of the present invention bind to antigenic polypeptides or proteins or protein fragments that are present and specifically associated with strains of *Neisseria meningitidis* MenB. In some instances, these antigens may be associated with specific strains, such as those antigens specific for the MenB strains. The antibodies of the invention may be immobilized to a matrix and utilized in an immunoassay or on an affinity chromatography column, to enable the detection and/or separation of polypeptides, proteins or protein fragments or cells comprising such polypeptides, proteins or protein fragments. Alternatively, such polypeptides, proteins or protein fragments may be immobilized so as to detect antibodies bindably specific thereto.

Antibodies to the proteins of the invention, both polyclonal and monoclonal, may be prepared by conventional methods. In general, the protein is first used to immunize a suitable animal, preferably a mouse, rat, rabbit or goat. Rabbits and goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies. Immunization is generally performed by mixing or emulsifying the protein in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 µg/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by in vitro immunization using methods known in the art, which for the purposes of this

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invention is considered equivalent to *in vivo* immunization. Polyclonal antisera is obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating at 4°C for 2-18 hours. The serum is recovered by centrifugation (e.g., 1,000g for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

Monoclonal antibodies are prepared using the standard method of Kohler & Milstein (*Nature* (1975) 256:495-96), or a modification thereof. Typically, a mouse or rat is immunized as described above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate or well coated with the protein antigen. B-cells that express membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (e.g., hypoxanthine, aminopterin, thymidine medium, "HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected MAb-secreting hybridomas are then cultured either *in vitro* (e.g., in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly ^{32}P and ^{125}I), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. "Specific binding partner" refers to a protein capable of binding a ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific therefor. Other specific binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various

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labels into distinct classes, as the same label may serve in several different modes. For example, ^{125}I may serve as a radioactive label or as an electron-dense reagent. HRP may serve as enzyme or as antigen for a MAb. Further, one may combine various labels for desired effect. For example, MAbs and avidin also require labels in the practice of this invention: thus, one might label a MAb with biotin, and detect its presence with avidin labeled with ^{125}I , or with an anti-biotin MAb labeled with HRP. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

Antigens, immunogens, polypeptides, proteins or protein fragments of the present invention elicit formation of specific binding partner antibodies. These antigens, immunogens, polypeptides, proteins or protein fragments of the present invention comprise immunogenic compositions of the present invention. Such immunogenic compositions may further comprise or include adjuvants, carriers, or other compositions that promote or enhance or stabilize the antigens, polypeptides, proteins or protein fragments of the present invention. Such adjuvants and carriers will be readily apparent to those of ordinary skill in the art.

Pharmaceutical Compositions

Pharmaceutical compositions can include either polypeptides, antibodies, or nucleic acid of the invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature, when given to a patient that is febrile. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in

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advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgment of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

Delivery Methods

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be treated.

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Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal and transcutaneous applications, needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Vaccines

Vaccines according to the invention may either be prophylactic (i.e., to prevent infection) or therapeutic (i.e., to treat disease after infection).

Such vaccines comprise immunizing antigen(s) or immunogen(s), immunogenic polypeptide, protein(s) or protein fragments, or nucleic acids (e.g., ribonucleic acid or deoxyribonucleic acid), usually in combination with "pharmaceutically acceptable carriers," which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the immunogen or antigen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, *H. pylori*, etc. pathogens.

Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59 (PCT Publ. No. WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a

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larger particle size emulsion, and (c) RibiTM adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (DetoxTM); (3) saponin adjuvants, such as StimulonTM (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (e.g., IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (e.g., gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), *etc.*; (6) detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT), a pertussis toxin (PT), or an *E. coli* heat-labile toxin (LT), particularly LT-K63, LT-R72, CT-S109, PT-K9/G129; see, e.g., WO 93/13302 and WO 92/19265; and (7) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum and MF59 are preferred.

As mentioned above, muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), *etc.*

The vaccine compositions comprising immunogenic compositions (e.g., which may include the antigen, pharmaceutically acceptable carrier, and adjuvant) typically will contain diluents, such as water, saline, glycerol, ethanol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Alternatively, vaccine compositions comprising immunogenic compositions may comprise an antigen, polypeptide, protein, protein fragment or nucleic acid in a pharmaceutically acceptable carrier.

More specifically, vaccines comprising immunogenic compositions comprise an immunologically effective amount of the immunogenic polypeptides, as well as any other of the above-mentioned components, as needed. By "immunologically effective amount", it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of

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individual to be treated (e.g., nonhuman primate, primate, *etc.*), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

Typically, the vaccine compositions or immunogenic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect, as discussed above under pharmaceutically acceptable carriers.

The immunogenic compositions are conventionally administered parenterally, e.g., by injection, either subcutaneously or intramuscularly. Additional formulations suitable for other modes of administration include oral and pulmonary formulations, suppositories, and transdermal and transcutaneous applications. Dosage treatment may be a single dose schedule or a multiple dose schedule. The vaccine may be administered in conjunction with other immunoregulatory agents.

As an alternative to protein-based vaccines, DNA vaccination may be employed (e.g., Robinson & Torres (1997) *Seminars in Immunology* 9:271-283; Donnelly *et al.* (1997) *Annu Rev Immunol* 15:617-648).

Gene Delivery Vehicles

Gene therapy vehicles for delivery of constructs, including a coding sequence of a therapeutic of the invention, to be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches in *in vivo* or *ex vivo* modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence *in vivo* can be either constitutive or regulated.

The invention includes gene delivery vehicles capable of expressing the contemplated nucleic acid sequences. The gene delivery vehicle is preferably a viral vector and, more preferably, a retroviral, adenoviral, adeno-associated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus,

paramyxovirus, parvovirus, picornavirus, poxvirus, or togavirus viral vector. See generally, Jolly (1994) *Cancer Gene Therapy* 1:51-64; Kimura (1994) *Human Gene Therapy* 5:845-852; Connelly (1995) *Human Gene Therapy* 6:185-193; and Kaplitt (1994) *Nature Genetics* 6:148-153.

Retroviral vectors are well known in the art, including B, C and D type retroviruses, xenotropic retroviruses (for example, NZB-X1, NZB-X2 and NZB9-1 (see O'Neill (1985) *J. Virol.* 53:160) polytropic retroviruses e.g., MCF and MCF-MLV (see Kelly (1983) *J. Virol.* 45:291), spumaviruses and lentiviruses. See RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985.

Portions of the retroviral gene therapy vector may be derived from different retroviruses. For example, retrovector LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site from a Rous Sarcoma Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.

These recombinant retroviral vectors may be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (see US patent 5,591,624). Retrovirus vectors can be constructed for site-specific integration into host cell DNA by incorporation of a chimeric integrase enzyme into the retroviral particle (see WO96/37626). It is preferable that the recombinant viral vector is a replication defective recombinant virus.

Packaging cell lines suitable for use with the above-described retrovirus vectors are well known in the art, are readily prepared (see WO95/30763 and WO92/05266), and can be used to create producer cell lines (also termed vector cell lines or "VCLs") for the production of recombinant vector particles. Preferably, the packaging cell lines are made from human parent cells (e.g., HT1080 cells) or mink parent cell lines, which eliminates inactivation in human serum.

Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia, Virus, Murine Leukemia Virus, Mink-Cell Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley and Rowe (1976) *J Virol* 19:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC No. VR-590), Kirsten, Harvey Sarcoma Virus and

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Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-190). Such retroviruses may be obtained from depositories or collections such as the American Type Culture Collection ("ATCC") in Rockville, Maryland or isolated from known sources using commonly available techniques.

Exemplary known retroviral gene therapy vectors employable in this invention include those described in patent applications GB2200651, EP0415731, EP0345242, EP0334301, WO89/02468; WO89/05349, WO89/09271, WO90/02806, WO90/07936, WO94/03622, WO93/25698, WO93/25234, WO93/11230, WO93/10218, WO91/02805, WO91/02825, WO95/07994, US 5,219,740, US 4,405,712, US 4,861,719, US 4,980,289, US 4,777,127, US 5,591,624. See also Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53 (1993) 83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

Human adenoviral gene therapy vectors are also known in the art and employable in this invention. See, for example, Berkner (1988) *Biotechniques* 6:616 and Rosenfeld (1991) *Science* 252:431, and WO93/07283, WO93/06223, and WO93/07282. Exemplary known adenoviral gene therapy vectors employable in this invention include those described in the above referenced documents and in WO94/12649, WO93/03769, WO93/19191, WO94/28938, WO95/11984, WO95/00655, WO95/27071, WO95/29993, WO95/34671, WO96/05320, WO94/08026, WO94/11506, WO93/06223, WO94/24299, WO95/14102, WO95/24297, WO95/02697, WO94/28152, WO94/24299, WO95/09241, WO95/25807, WO95/05835, WO94/18922 and WO95/09654. Alternatively, administration of DNA linked to killed adenovirus as described in Curiel (1992) *Hum. Gene Ther.* 3:147-154 may be employed. The gene delivery vehicles of the invention also include adenovirus associated virus (AAV) vectors. Leading and preferred examples of such vectors for use in this invention are the AAV-2 based vectors disclosed in Srivastava, WO93/09239. Most preferred AAV vectors comprise the two AAV inverted terminal repeats in which the native D-sequences are modified by substitution of nucleotides, such that at least 5 native nucleotides and up to 18 native nucleotides, preferably at least 10 native nucleotides up to 18 native nucleotides, most preferably 10 native nucleotides are retained and the remaining nucleotides of the D-sequence are deleted or replaced with non-native nucleotides. The native

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D-sequences of the AAV inverted terminal repeats are sequences of 20 consecutive nucleotides in each AAV inverted terminal repeat (i.e., there is one sequence at each end) which are not involved in HP formation. The non-native replacement nucleotide may be any nucleotide other than the nucleotide found in the native D-sequence in the same position. Other employable exemplary AAV vectors are pWP-19, pWN-1, both of which are disclosed in Nahreini (1993) *Gene* 124:257-262. Another example of such an AAV vector is psub201 (see Samulski (1987) *J. Virol.* 61:3096). Another exemplary AAV vector is the Double-D ITR vector. Construction of the Double-D ITR vector is disclosed in US Patent 5,478,745. Still other vectors are those disclosed in Carter US Patent 4,797,368 and Muzyczka US Patent 5,139,941, Chartejee US Patent 5,474,935, and Kotin WO94/288157. Yet a further example of an AAV vector employable in this invention is SSV9AFABTKneo, which contains the AFP enhancer and albumin promoter and directs expression predominantly in the liver. Its structure and construction are disclosed in Su (1996) *Human Gene Therapy* 7:463-470. Additional AAV gene therapy vectors are described in US 5,354,678, US 5,173,414, US 5,139,941, and US 5,252,479.

The gene therapy vectors comprising sequences of the invention also include herpes vectors. Leading and preferred examples are herpes simplex virus vectors containing a sequence encoding a thymidine kinase polypeptide such as those disclosed in US 5,288,641 and EP0176170 (Roizman). Additional exemplary herpes simplex virus vectors include HFEM/ICP6-LacZ disclosed in WO95/04139 (Wistar Institute), pHSVlac described in Geller (1988) *Science* 241:1667-1669 and in WO90/09441 and WO92/07945, HSV Us3::pgC-lacZ described in Fink (1992) *Human Gene Therapy* 3:11-19 and HSV 7134, 2 RH 105 and GAL4 described in EP 0453242 (Breakefield), and those deposited with the ATCC as accession numbers ATCC VR-977 and ATCC VR-260.

Also contemplated are alpha virus gene therapy vectors that can be employed in this invention. Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in US patents 5,091,309, 5,217,879, and WO92/10578. More particularly, those alpha virus vectors described in U.S. Serial No. 08/405,627, filed March 15, 1995, WO94/21792, WO92/10578,

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WO95/07994, US 5,091,309 and US 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in Rockville, Maryland or isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (see USSN 08/679640).

DNA vector systems such as eukaryotic layered expression systems are also useful for expressing the nucleic acids of the invention. See WO95/07994 for a detailed description of eukaryotic layered expression systems. Preferably, the eukaryotic layered expression systems of the invention are derived from alphavirus vectors and most preferably from Sindbis viral vectors.

Other viral vectors suitable for use in the present invention include those derived from poliovirus, for example ATCC VR-58 and those described in Evans, *Nature* 339 (1989) 385 and Sabin (1973) *J. Biol. Standardization* 1:115; rhinovirus, for example ATCC VR-1110 and those described in Arnold (1990) *J Cell Biochem* L401; pox viruses such as canary pox virus or vaccinia virus, for example ATCC VR-111 and ATCC VR-2010 and those described in Fisher-Hoch (1989) *Proc Natl Acad Sci* 86:317; Flexner (1989) *Ann NY Acad Sci* 569:86, Flexner (1990) *Vaccine* 8:17; in US 4,603,112 and US 4,769,330 and WO89/01973; SV40 virus, for example ATCC VR-305 and those described in Mulligan (1979) *Nature* 277:108 and Madzak (1992) *J Gen Virol* 73:1533; influenza virus, for example ATCC VR-797 and recombinant influenza viruses made employing reverse genetics techniques as described in US 5,166,057 and in Enami (1990) *Proc Natl Acad Sci* 87:3802-3805; Enami & Palese (1991) *J Virol* 65:2711-2713 and Luytjes (1989) *Cell* 59:110, (see also McMichael (1983) *NEJ Med* 309:13, and Yap (1978) *Nature* 273:238 and *Nature* (1979) 277:108); human immunodeficiency virus as described in EP-0386882 and in Buchschacher (1992) *J. Virol.* 66:2731; measles virus, for example ATCC VR-67 and VR-1247 and those described in EP-0440219; Aura virus, for example ATCC VR-368; Bebaru virus, for example ATCC VR-600 and ATCC VR-1240; Cabassou virus, for example ATCC VR-922; Chikungunya virus, for example ATCC VR-64 and ATCC VR-1241; Fort Morgan Virus, for example ATCC VR-924; Getah virus, for example ATCC VR-369 and ATCC VR-1243; Kyzylagach virus, for example ATCC VR-927; Mayaro virus, for example ATCC VR-66; Mucambo virus, for example ATCC VR-580 and ATCC VR-1244; Ndumu virus, for example ATCC VR-371; Pixuna virus, for example ATCC VR-372 and ATCC VR-1245; Tonate virus, for example

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ATCC VR-925; Trinit virus, for example ATCC VR-469; Una virus, for example ATCC VR-374; Whataroa virus, for example ATCC VR-926; Y-62-33 virus, for example ATCC VR-375; O'Nyong virus, Eastern encephalitis virus, for example ATCC VR-65 and ATCC VR-1242; Western encephalitis virus, for example ATCC VR-70, ATCC VR-1251, ATCC VR-622 and ATCC VR-1252; and coronavirus, for example ATCC VR-740 and those described in Hamre (1966) *Proc Soc Exp Biol Med* 121:190.

Delivery of the compositions of this invention into cells is not limited to the above mentioned viral vectors. Other delivery methods and media may be employed such as, for example, nucleic acid expression vectors, polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example see US Serial No. 08/366,787, filed December 30, 1994 and Curiel (1992) *Hum Gene Ther* 3:147-154 ligand linked DNA, for example see Wu (1989) *J Biol Chem* 264:16985-16987, eucaryotic cell delivery vehicles cells, for example see US Serial No. 08/240,030, filed May 9, 1994, and US Serial No. 08/404,796, deposition of photopolymerized hydrogel materials, hand-held gene transfer particle gun, as described in US Patent 5,149,655, ionizing radiation as described in US5,206,152 and in WO92/11033, nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip (1994) *Mol Cell Biol* 14:2411-2418 and in Woffendin (1994) *Proc Natl Acad Sci* 91:1581-1585.

Particle mediated gene transfer may be employed, for example see US Serial No. 60/023,867. Briefly, the sequence can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, as described in Wu & Wu (1987) *J. Biol. Chem.* 262:4429-4432, insulin as described in Hucked (1990) *Biochem Pharmacol* 40:253-263, galactose as described in Plank (1992) *Bioconjugate Chem* 3:533-539, lactose or transferrin.

Naked DNA may also be employed to transform a host cell. Exemplary naked DNA introduction methods are described in WO 90/11092 and US 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved

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further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm.

Liposomes that can act as gene delivery vehicles are described in U.S. 5,422,120, WO95/13796, WO94/23697, WO91/14445 and EP-524,968. As described in USSN. 60/023,867, on non-viral delivery, the nucleic acid sequences encoding a polypeptide can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then be incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, insulin, galactose, lactose, or transferrin. Other delivery systems include the use of liposomes to encapsulate DNA comprising the gene under the control of a variety of tissue-specific or ubiquitously-active promoters. Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al* (1994) *Proc. Natl. Acad. Sci. USA* 91(24):11581-11585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in U.S. 5,149,655; use of ionizing radiation for activating transferred gene, as described in U.S. 5,206,152 and WO92/11033

Exemplary liposome and polycationic gene delivery vehicles are those described in US 5,422,120 and 4,762,915; in WO 95/13796; WO94/23697; and WO91/14445; in EP-0524968; and in Stryer, *Biochemistry*, pages 236-240 (1975) W.H. Freeman, San Francisco; Szoka (1980) *Biochem Biophys Acta* 600:1; Bayer (1979) *Biochem Biophys Acta* 550:464; Rivnay (1987) *Meth Enzymol* 149:119; Wang (1987) *Proc Natl Acad Sci* 84:7851; Plant (1989) *Anal Biochem* 176:420.

A polynucleotide composition can comprise a therapeutically effective amount of a gene therapy vehicle, as the term is defined above. For purposes of the present invention, an effective dose will be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

Delivery Methods

Once formulated, the polynucleotide compositions of the invention can be administered (1) directly to the subject; (2) delivered *ex vivo*, to cells derived from the subject; or (3) *in vitro* for expression of recombinant proteins. The subjects to be treated can be mammals or birds. Also, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, transdermally or transcutaneously, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a tumor or lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal applications, needles, and gene guns or hypodermic sprays. Dosage treatment may be a single dose schedule or a multiple dose schedule. See WO98/20734.

Methods for the *ex vivo* delivery and reimplantation of transformed cells into a subject are known in the art and described in e.g., WO93/14778. Examples of cells useful in *ex vivo* applications include, for example, stem cells, particularly hematopoietic, lymph cells, macrophages, dendritic cells, or tumor cells.

Generally, delivery of nucleic acids for both *ex vivo* and *in vitro* applications can be accomplished by the following procedures, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

Polynucleotide and Polypeptide pharmaceutical compositions

In addition to the pharmaceutically acceptable carriers and salts described above, the following additional agents can be used with polynucleotide and/or polypeptide compositions.

A. Polypeptides

One example are polypeptides which include, without limitation: asialoorosomucoid (ASOR); transferrin; asialoglycoproteins; antibodies; antibody fragments; ferritin; interleukins; interferons, granulocyte, macrophage colony stimulating factor (GM-CSF),

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granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor and erythropoietin. Viral antigens, such as envelope proteins, can also be used. Also, proteins from other invasive organisms, such as the 17 amino acid peptide from the circumsporozoite protein of *plasmodium falciparum* known as RII.

B. Hormones, Vitamins, Etc.

Other groups that can be included in a pharmaceutical composition include, for example: hormones, steroids, androgens, estrogens, thyroid hormone, or vitamins, folic acid.

C. Polyalkylenes, Polysaccharides, etc.

Also, polyalkylene glycol can be included in a pharmaceutical compositions with the desired polynucleotides and/or polypeptides. In a preferred embodiment, the polyalkylene glycol is polyethylene glycol. In addition, mono-, di-, or polysaccharides can be included. In a preferred embodiment of this aspect, the polysaccharide is dextran or DEAE-dextran. Also, chitosan and poly(lactide-co-glycolide) may be included in a pharmaceutical composition.

D. Lipids, and Liposomes

The desired polynucleotide or polypeptide can also be encapsulated in lipids or packaged in liposomes prior to delivery to the subject or to cells derived therefrom.

Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid or polypeptide. The ratio of condensed polynucleotide to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight (1991) *Biochim. Biophys. Acta.* 1097:1-17; Straubinger (1983) *Meth. Enzymol.* 101:512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7416); mRNA (Malone (1989) *Proc. Natl. Acad. Sci. USA* 86:6077-6081); and purified transcription factors (Debs (1990) *J. Biol. Chem.* 265:10189-10192), in functional form.

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Cationic liposomes are readily available. For example, N(1-2,3-dioleoyloxy)propyl)-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner *supra*). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g., Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; WO90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See e.g., Straubinger (1983) *Meth. Immunol.* 101:512-527; Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; Papahadjopoulos (1975) *Biochim. Biophys. Acta* 394:483; Wilson (1979) *Cell* 17:77; Deamer & Bangham (1976) *Biochim. Biophys. Acta* 443:629; Ostro (1977) *Biochem. Biophys. Res. Commun.* 76:836; Fraley (1979) *Proc. Natl. Acad. Sci. USA* 76:3348; Enoch & Strittmatter (1979) *Proc. Natl. Acad. Sci. USA* 76:145; Fraley (1980) *J. Biol. Chem.* (1980) 255:10431; Szoka & Papahadjopoulos (1978) *Proc. Natl. Acad. Sci. USA* 75:145; and Schaefer-Ridder (1982) *Science* 215:166.

E. Lipoproteins

In addition, lipoproteins can be included with the polynucleotide or polypeptide to be delivered. Examples of lipoproteins to be utilized include: chylomicrons, HDL, IDL, LDL, and VLDL. Mutants, fragments, or fusions of these proteins can also be used. Also, modifications of naturally occurring lipoproteins can be used, such as acetylated LDL. These

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lipoproteins can target the delivery of polynucleotides to cells expressing lipoprotein receptors. Preferably, if lipoproteins are including with the polynucleotide to be delivered, no other targeting ligand is included in the composition.

Naturally occurring lipoproteins comprise a lipid and a protein portion. The protein portion are known as apoproteins. At the present, apoproteins A, B, C, D, and E have been isolated and identified. At least two of these contain several proteins, designated by Roman numerals, AI, AII, AIV; CI, CII, CIII.

A lipoprotein can comprise more than one apoprotein. For example, naturally occurring chylomicrons comprises of A, B, C, and E; over time these lipoproteins lose A and acquire C and E apoproteins. VLDL comprises A, B, C, and E apoproteins, LDL comprises apoprotein B; and HDL comprises apoproteins A, C, and E.

The amino acid sequences of these apoproteins are known and are described in, for example, Breslow (1985) *Annu Rev. Biochem* 54:699; Law (1986) *Adv. Exp Med. Biol.* 151:162; Chen (1986) *J Biol Chem* 261:12918; Kane (1980) *Proc Natl Acad Sci USA* 77:2465; and Utermann (1984) *Hum Genet* 65:232.

Lipoproteins contain a variety of lipids including, triglycerides, cholesterol (free and esters), and phospholipids. The composition of the lipids varies in naturally occurring lipoproteins. For example, chylomicrons comprise mainly triglycerides. A more detailed description of the lipid content of naturally occurring lipoproteins can be found, for example, in *Meth. Enzymol.* 128 (1986). The composition of the lipids are chosen to aid in conformation of the apoprotein for receptor binding activity. The composition of lipids can also be chosen to facilitate hydrophobic interaction and association with the polynucleotide binding molecule.

Naturally occurring lipoproteins can be isolated from serum by ultracentrifugation, for instance. Such methods are described in *Meth. Enzymol. (supra)*; Pitas (1980) *J. Biochem.* 255:5454-5460 and Mahey (1979) *J Clin. Invest* 64:743-750.

Lipoproteins can also be produced by *in vitro* or recombinant methods by expression of the apoprotein genes in a desired host cell. See, for example, Atkinson (1986) *Annu Rev Biophys Chem* 15:403 and Radding (1958) *Biochim Biophys Acta* 30: 443.

Lipoproteins can also be purchased from commercial suppliers, such as Biomedical Technologies, Inc., Stoughton, Massachusetts, USA.

Further description of lipoproteins can be found in Zuckermann et al., PCT. Appln. No. US97/14465.

F. Polycationic Agents

Polycationic agents can be included, with or without lipoprotein, in a composition with the desired polynucleotide and/or polypeptide to be delivered.

Polycationic agents, typically, exhibit a net positive charge at physiological relevant pH and are capable of neutralizing the electrical charge of nucleic acids to facilitate delivery to a desired location. These agents have both in vitro, ex vivo, and in vivo applications. Polycationic agents can be used to deliver nucleic acids to a living subject either intramuscularly, subcutaneously, etc.

The following are examples of useful polypeptides as polycationic agents: polylysine, polyarginine, polyornithine, and protamine. Other examples of useful polypeptides include histones, protamines, human serum albumin, DNA binding proteins, non-histone chromosomal proteins, coat proteins from DNA viruses, such as Φ X174, transcriptional factors also contain domains that bind DNA and therefore may be useful as nucleic acid condensing agents. Briefly, transcriptional factors such as C/CEBP, c-jun, c-fos, AP-1, AP-2, AP-3, CPF, Prot-1, Sp-1, Oct-1, Oct-2, CREP, and TFIID contain basic domains that bind DNA sequences.

Organic polycationic agents include: spermine, spermidine, and putrescine.

The dimensions and of the physical properties of a polycationic agent can be extrapolated from the list above, to construct other polypeptide polycationic agents or to produce synthetic polycationic agents.

G. Synthetic Polycationic Agents

Synthetic polycationic agents which are useful in pharmaceutical compositions include, for example, DEAE-dextran, polybrene. Lipofectin™, and lipofectAMINE™ are monomers that form polycationic complexes when combined with polynucleotides or polypeptides.

Immunodiagnostic Assays

Neisseria MenB antigens, or antigenic fragments thereof, of the invention can be used in immunoassays to detect antibody levels (or, conversely, anti-*Neisseria* MenB antibodies can be used to detect antigen levels). Immunoassays based on well defined, recombinant antigens can be developed to replace invasive diagnostics methods. Antibodies to *Neisseria* MenB proteins or fragments thereof within biological samples, including for example, blood or serum samples, can be detected. Design of the immunoassays is subject to a great deal of variation, and a variety of these are known in the art. Protocols for the immunoassay may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the compositions of the invention, in suitable containers, along with the remaining reagents and materials (for example, suitable buffers, salt solutions, *etc.*) required for the conduct of the assay, as well as suitable set of assay instructions.

Nucleic Acid Hybridization

"Hybridization" refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Typically, one sequence will be fixed to a solid support and the other will be free in solution. Then, the two sequences will be placed in contact with one another under conditions that favor hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase sequence to the solid support (Denhardt's reagent or BLOTTO); concentration of the sequences; use of compounds to increase the rate of association of sequences (dextran sulfate or polyethylene glycol); and the

stringency of the washing conditions following hybridization. See Sambrook *et al.* (*supra*) Volume 2, chapter 9, pages 9.47 to 9.57.

"Stringency" refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. For example, the combination of temperature and salt concentration should be chosen that is approximately 120 to 200°C below the calculated T_m of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the sequence of interest and then washed under conditions of different stringencies. See Sambrook *et al.* at page 9.50.

Variables to consider when performing, for example, a Southern blot are (1) the complexity of the DNA being blotted and (2) the homology between the probe and the sequences being detected. The total amount of the fragment(s) to be studied can vary a magnitude of 10, from 0.1 to 1 µg for a plasmid or phage digest to 10^{-9} to 10^{-8} g for a single copy gene in a highly complex eukaryotic genome. For lower complexity polynucleotides, substantially shorter blotting, hybridization, and exposure times, a smaller amount of starting polynucleotides, and lower specific activity of probes can be used. For example, a single-copy yeast gene can be detected with an exposure time of only 1 hour starting with 1 µg of yeast DNA, blotting for two hours, and hybridizing for 4-8 hours with a probe of 10^8 cpm/µg. For a single-copy mammalian gene a conservative approach would start with 10 µg of DNA, blot overnight, and hybridize overnight in the presence of 10% dextran sulfate using a probe of greater than 10^8 cpm/µg, resulting in an exposure time of ~24 hours.

Several factors can affect the melting temperature (T_m) of a DNA-DNA hybrid between the probe and the fragment of interest, and consequently, the appropriate conditions for hybridization and washing. In many cases the probe is not 100% homologous to the fragment. Other commonly encountered variables include the length and total G+C content of the hybridizing sequences and the ionic strength and formamide content of the hybridization buffer. The effects of all of these factors can be approximated by a single equation:

$$T_m = 81 + 16.6(\log_{10} C_i) + 0.4(\%(G + C)) - 0.6(\%\text{formamide}) - 600/n - 1.5(\%\text{mismatch})$$

where C_i is the salt concentration (monovalent ions) and n is the length of the hybrid in base pairs (slightly modified from Meinkoth & Wahl (1984) *Anal. Biochem.* 138:267-284).

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In designing a hybridization experiment, some factors affecting nucleic acid hybridization can be conveniently altered. The temperature of the hybridization and washes and the salt concentration during the washes are the simplest to adjust. As the temperature of the hybridization increases (i.e., stringency), it becomes less likely for hybridization to occur between strands that are nonhomologous, and as a result, background decreases. If the radiolabeled probe is not completely homologous with the immobilized fragment (as is frequently the case in gene family and interspecies hybridization experiments), the hybridization temperature must be reduced, and background will increase. The temperature of the washes affects the intensity of the hybridizing band and the degree of background in a similar manner. The stringency of the washes is also increased with decreasing salt concentrations.

In general, convenient hybridization temperatures in the presence of 50% formamide are 42°C for a probe with is 95% to 100% homologous to the target fragment, 37°C for 90% to 95% homology, and 32°C for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If non-specific bands or high background are observed after autoradiography, the filter can be washed at high stringency and reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

Nucleic Acid Probe Assays

Methods such as PCR, branched DNA probe assays, or blotting techniques utilizing nucleic acid probes according to the invention can determine the presence of cDNA or mRNA. A probe is said to "hybridize" with a sequence of the invention if it can form a duplex or double stranded complex, which is stable enough to be detected.

The nucleic acid probes will hybridize to the Neisserial nucleotide sequences of the invention (including both sense and antisense strands). Though many different nucleotide sequences will encode the amino acid sequence, the native Neisserial sequence is preferred because it is the actual sequence present in cells. mRNA represents a coding sequence and so

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a probe should be complementary to the coding sequence; single-stranded cDNA is complementary to mRNA, and so a cDNA probe should be complementary to the non-coding sequence.

The probe sequence need not be identical to the Neisserial sequence (or its complement) -- some variation in the sequence and length can lead to increased assay sensitivity if the nucleic acid probe can form a duplex with target nucleotides, which can be detected. Also, the nucleic acid probe can include additional nucleotides to stabilize the formed duplex. Additional Neisserial sequence may also be helpful as a label to detect the formed duplex. For example, a non-complementary nucleotide sequence may be attached to the 5' end of the probe, with the remainder of the probe sequence being complementary to a Neisserial sequence. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided that the probe sequence has sufficient complementarity with the a Neisserial sequence in order to hybridize therewith and thereby form a duplex which can be detected.

The exact length and sequence of the probe will depend on the hybridization conditions, such as temperature, salt condition and the like. For example, for diagnostic applications, depending on the complexity of the analyte sequence, the nucleic acid probe typically contains at least 10-20 nucleotides, preferably 15-25, and more preferably at least 30 nucleotides, although it may be shorter than this. Short primers generally require cooler temperatures to form sufficiently stable hybrid complexes with the template.

Probes may be produced by synthetic procedures, such as the triester method of Matteucci *et al.* (*J. Am. Chem. Soc.* (1981) 103:3185), or according to Urdea *et al.* (*Proc. Natl. Acad. Sci. USA* (1983) 80: 7461), or using commercially available automated oligonucleotide synthesizers.

The chemical nature of the probe can be selected according to preference. For certain applications, DNA or RNA are appropriate. For other applications, modifications may be incorporated e.g., backbone modifications, such as phosphorothioates or methylphosphonates, can be used to increase *in vivo* half-life, alter RNA affinity, increase nuclease resistance *etc.* (e.g., see Agrawal & Iyer (1995) *Curr Opin Biotechnol* 6:12-19; Agrawal (1996) *TIBTECH* 14:376-387); analogues such as peptide nucleic acids may also be

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used (e.g., see Corey (1997) *TIBTECH* 15:224-229; Buchardt *et al.* (1993) *TIBTECH* 11:384-386).

One example of a nucleotide hybridization assay is described by Urdea *et al.* in international patent application WO92/02526 (see also U.S. Patent 5,124,246).

Alternatively, the polymerase chain reaction (PCR) is another well-known means for detecting small amounts of target nucleic acids. The assay is described in: Mullis *et al.* (*Meth. Enzymol.* (1987) 155: 335-350); US patent 4,683,195; and US patent 4,683,202. Two "primer" nucleotides hybridize with the target nucleic acids and are used to prime the reaction. The primers can comprise sequence that does not hybridize to the sequence of the amplification target (or its complement) to aid with duplex stability or, for example, to incorporate a convenient restriction site. Typically, such sequence will flank the desired Neisserial sequence.

A thermostable polymerase creates copies of target nucleic acids from the primers using the original target nucleic acids as a template. After a threshold amount of target nucleic acids are generated by the polymerase, they can be detected by more traditional methods, such as Southern blots. When using the Southern blot method, the labeled probe will hybridize to the Neisserial sequence (or its complement).

Also, mRNA or cDNA can be detected by traditional blotting techniques described in Sambrook *et al* (*supra*). mRNA, or cDNA generated from mRNA using a polymerase enzyme, can be purified and separated using gel electrophoresis. The nucleic acids on the gel are then blotted onto a solid support, such as nitrocellulose. The solid support is exposed to a labeled probe and then washed to remove any unhybridized probe. Next, the duplexes containing the labeled probe are detected. Typically, the probe is labeled with a radioactive moiety.

EXAMPLES

The invention is based on the 961 nucleotide sequences from the genome of *N. meningitidis* set out in Appendix C, SEQ ID NOs:1-961 of the '573 application, which together represent substantially the complete genome of serotype B of *N. meningitidis*, as well as the full length genome sequence shown in Appendix D, SEQ ID NO 1068 of the '573

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application, and the full length genome sequence shown in Appendix A hereto, SEQ ID NO. 1.

It will be self-evident to the skilled person how this sequence information can be utilized according to the invention, as above described.

The standard techniques and procedures which may be employed in order to perform the invention (e.g. to utilize the disclosed sequences to predict polypeptides useful for vaccination or diagnostic purposes) were summarized above. This summary is not a limitation on the invention but, rather, gives examples that may be used, but are not required.

These sequences are derived from contigs shown in Appendix C (SEQ ID NOs 1-961) and from the full length genome sequence shown in Appendix D (SEQ ID NO 1068), which were prepared during the sequencing of the genome of *N. meningitidis* (strain B). The full length sequence was assembled using the TIGR Assembler as described by G.S. Sutton et al., *TIGR Assembler: A New Tool for Assembling Large Shotgun Sequencing Projects*, Genome Science and Technology, 1:9-19 (1995) [see also R. D. Fleischmann, et al., Science 269, 496-512 (1995); C. M. Fraser, et al., Science 270, 397-403 (1995); C. J. Bult, et al., Science 273, 1058-73 (1996); C. M. Fraser, et al., Nature 390, 580-586 (1997); J.-F. Tomb, et al., Nature 388, 539-547 (1997); H. P. Klenk, et al., Nature 390, 364-70 (1997); C. M. Fraser, et al., Science 281, 375-88 (1998); M. J. Gardner, et al., Science 282, 1126-1132 (1998); K. E. Nelson, et al., Nature 399, 323-9 (1999)]. Then, using the above-described methods, putative translation products of the sequences were determined. Computer analysis of the translation products were determined based on database comparisons. Corresponding gene and protein sequences, if any, were identified in *Neisseria meningitidis* (Strain A) and *Neisseria gonorrhoeae*. Then the proteins were expressed, purified, and characterized to assess their antigenicity and immunogenicity.

In particular, the following methods were used to express, purify, and biochemically characterize the proteins of the invention.

Chromosomal DNA Preparation

N. meningitidis strain 2996 was grown to exponential phase in 100 ml of GC medium, harvested by centrifugation, and resuspended in 5 ml buffer (20% Sucrose, 50 mM Tris-HCl, 50 mM EDTA, adjusted to pH 8.0). After 10 minutes incubation on ice, the bacteria were

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lysed by adding 10 ml lysis solution (50 mM NaCl, 1% Na-Sarkosyl, 50 µg/ml Proteinase K), and the suspension was incubated at 37°C for 2 hours. Two phenol extractions (equilibrated to pH 8) and one ChCl_3 /isoamylalcohol (24:1) extraction were performed. DNA was precipitated by addition of 0.3M sodium acetate and 2 volumes ethanol, and was collected by centrifugation. The pellet was washed once with 70% ethanol and redissolved in 4 ml buffer (10 mM Tris-HCl, 1mM EDTA, pH 8). The DNA concentration was measured by reading the OD at 260 nm.

Oligonucleotide design

Synthetic oligonucleotide primers were designed on the basis of the coding sequence of each ORF, using (a) the meningococcus B sequence when available, or (b) the gonococcus/meningococcus A sequence, adapted to the codon preference usage of meningococcus. Any predicted signal peptides were omitted, by deducing the 5'-end amplification primer sequence immediately downstream from the predicted leader sequence.

For most ORFs, the 5' primers included two restriction enzyme recognition sites (*Bam*HI-*Nde*I, *Bam*HI-*Nhe*I, or *Eco*RI-*Nhe*I, depending on the gene's restriction pattern); the 3' primers included a *Xho*I restriction site. This procedure was established in order to direct the cloning of each amplification product (corresponding to each ORF) into two different expression systems: pGEX-KG (using either *Bam*HI-*Xho*I or *Eco*RI-*Xho*I), and pET21b+ (using either *Nde*I-*Xho*I or *Nhe*I-*Xho*I).

5'-end primer tail:	<u>CGCGGATCCCATATG</u>	(<i>Bam</i> HI- <i>Nde</i> I)
	<u>CGCGGATCCGCTAGC</u>	(<i>Bam</i> HI- <i>Nhe</i> I)
	<u>CCGGAATTCTAGCTAGC</u>	(<i>Eco</i> RI- <i>Nhe</i> I)
3'-end primer tail:	<u>CCCGCTCGAG</u>	(<i>Xho</i> I)

For some ORFs, two different amplifications were performed to clone each ORF in the two expression systems. Two different 5' primers were used for each ORF; the same 3' *Xho*I primer was used as before:

5'-end primer tail:	<u>GGAATTCCCATATGGCCATGG</u>	(<i>Nde</i> I)
5'-end primer tail:	<u>CGGGATCC</u>	(<i>Bam</i> HI)

Other ORFs were cloned in the pTRC expression vector and expressed as an amino-terminus His-tag fusion. The predicted signal peptide may be included in the final product. *NheI*-*BamHI* restriction sites were incorporated using primers:

5'-end primer tail: GATCAGCTAGCCATATG (*NheI*)

3'-end primer tail: CGGGATCC (*BamHI*)

As well as containing the restriction enzyme recognition sequences, the primers included nucleotides which hybridized to the sequence to be amplified. The number of hybridizing nucleotides depended on the melting temperature of the whole primer, and was determined for each primer using the formulae:

$$T_m = 4 (G+C) + 2 (A+T) \quad (\text{tail excluded})$$

$$T_m = 64.9 + 0.41 (\% \text{ GC}) - 600/N \quad (\text{whole primer})$$

The average melting temperature of the selected oligos were 65-70°C for the whole oligo and 50-55°C for the hybridising region alone.

Oligos were synthesized by a Perkin Elmer 394 DNA/RNA Synthesizer, eluted from the columns in 2 ml $\text{NH}_4\text{-OH}$, and deprotected by 5 hours incubation at 56 °C. The oligos were precipitated by addition of 0.3M Na-Acetate and 2 volumes ethanol. The samples were then centrifuged and the pellets resuspended in either 100 μ l or 1ml of water. OD_{260} was determined using a Perkin Elmer Lambda Bio spectrophotometer and the concentration was determined and adjusted to 2-10 pmol/ μ l.

Table 1 shows the forward and reverse primers used for each amplification. In certain cases, it might be noted that the sequence of the primer does not exactly match the sequence in the ORF. When initial amplifications are performed, the complete 5' and/or 3' sequence may not be known for some meningococcal ORFs, although the corresponding sequences may have been identified in gonococcus. For amplification, the gonococcal sequences could thus be used as the basis for primer design, altered to take account of codon preference. In particular, the following codons may be changed: ATA \rightarrow ATT; TCG \rightarrow TCT; CAG \rightarrow CAA; AAG \rightarrow AAA; GAG \rightarrow GAA; CGA and CGG \rightarrow CGC; GGG \rightarrow GGC.

Amplification

The standard PCR protocol was as follows: 50-200 ng of genomic DNA were used as a template in the presence of 20-40 μ M of each oligo, 400-800 μ M dNTPs solution, 1x PCR

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buffer (including 1.5 mM MgCl₂), 2.5 units *TaqI* DNA polymerase (using Perkin-Elmer AmpliTaq, GIBCO Platinum, Pwo DNA polymerase, or Tahara Shuzo Taq polymerase).

In some cases, PCR was optimised by the addition of 10µl DMSO or 50 µl 2M betaine.

After a hot start (adding the polymerase during a preliminary 3 minute incubation of the whole mix at 95°C), each sample underwent a double-step amplification: the first 5 cycles were performed using as the hybridization temperature the one of the oligos excluding the restriction enzymes tail, followed by 30 cycles performed according to the hybridization temperature of the whole length oligos. The cycles were followed by a final 10 minute extension step at 72°C.

The standard cycles were as follows:

	Denaturation	Hybridisation	Elongation
First 5 cycles	30 seconds 95°C	30 seconds 50-55°C	30-60 seconds 72°C
Last 30 cycles	30 seconds 95°C	30 seconds 65-70°C	30-60 seconds 72°C

The elongation time varied according to the length of the ORF to be amplified.

The amplifications were performed using either a 9600 or a 2400 Perkin Elmer GeneAmp PCR System. To check the results, 1/10 of the amplification volume was loaded onto a 1-1.5% agarose gel and the size of each amplified fragment compared with a DNA molecular weight marker.

The amplified DNA was either loaded directly on a 1% agarose gel or first precipitated with ethanol and resuspended in a suitable volume to be loaded on a 1% agarose gel. The DNA fragment corresponding to the right size band was then eluted and purified from gel, using the Qiagen Gel Extraction Kit, following the instructions of the manufacturer. The final volume of the DNA fragment was 30µl or 50µl of either water or 10mM Tris, pH 8.5.

Digestion of PCR fragments

The purified DNA corresponding to the amplified fragment was split into 2 aliquots and double-digested with:

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NdeI/XhoI or *NheI/XhoI* for cloning into pET-21b+ and further expression of the protein as a C-terminus His-tag fusion

BamHI/XhoI or *EcoRI/XhoI* for cloning into pGEX-KG and further expression of the protein as a GST N-terminus fusion.

For ORF 76, *NheI/BamHI* for cloning into pTRC-HisA vector and further expression of the protein as N-terminus His-tag fusion.

Each purified DNA fragment was incubated (37°C for 3 hours to overnight) with 20 units of each restriction enzyme (New England Biolabs) in a either 30 or 40 µl final volume in the presence of the appropriate buffer. The digestion product was then purified using the QIAquick PCR purification kit, following the manufacturer's instructions, and eluted in a final volume of 30 (or 50) µl of either water or 10mM Tris-HCl, pH 8.5. The final DNA concentration was determined by 1% agarose gel electrophoresis in the presence of titrated molecular weight marker.

Digestion of the cloning vectors (pET22B, pGEX-KG and pTRC-His A)

10 µg plasmid was double-digested with 50 units of each restriction enzyme in 200 µl reaction volume in the presence of appropriate buffer by overnight incubation at 37°C. After loading the whole digestion on a 1% agarose gel, the band corresponding to the digested vector was purified from the gel using the Qiagen QIAquick Gel Extraction Kit and the DNA was eluted in 50 µl of 10 mM Tris-HCl, pH 8.5. The DNA concentration was evaluated by measuring OD₂₆₀ of the sample, and adjusted to 50 µg/µl. 1 µl of plasmid was used for each cloning procedure.

Cloning

The fragments corresponding to each ORF, previously digested and purified, were ligated in both pET22b and pGEX-KG. In a final volume of 20 µl, a molar ratio of 3:1 fragment/vector was ligated using 0.5 µl of NEB T4 DNA ligase (400 units/µl), in the presence of the buffer supplied by the manufacturer. The reaction was incubated at room temperature for 3 hours. In some experiments, ligation was performed using the Boehringer "Rapid Ligation Kit", following the manufacturer's instructions.

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In order to introduce the recombinant plasmid in a suitable strain, 100 μ l *E. coli* DH5 competent cells were incubated with the ligase reaction solution for 40 minutes on ice, then at 37°C for 3 minutes, then, after adding 800 μ l LB broth, again at 37°C for 20 minutes. The cells were then centrifuged at maximum speed in an Eppendorf microfuge and resuspended in approximately 200 μ l of the supernatant. The suspension was then plated on LB ampicillin (100 mg/ml).

The screening of the recombinant clones was performed by growing 5 randomly-chosen colonies overnight at 37 °C in either 2 ml (pGEX or pTC clones) or 5ml (pET clones) LB broth + 100 μ g/ml ampicillin. The cells were then pelleted and the DNA extracted using the Qiagen QIAprep Spin Miniprep Kit, following the manufacturer's instructions, to a final volume of 30 μ l. 5 μ l of each individual miniprep (approximately 1g) were digested with either *NdeI/XhoI* or *BamHI/XhoI* and the whole digestion loaded onto a 1-1.5% agarose gel (depending on the expected insert size), in parallel with the molecular weight marker (1Kb DNA Ladder, GIBCO). The screening of the positive clones was made on the base of the correct insert size.

Cloning

Certain ORFs may be cloned into the pGEX-HIS vector using *EcoRI-PstI*, *EcoRI-SalI*, or *SalI-PstI* cloning sites. After cloning, the recombinant plasmids may be introduced in the *E.coli* host W3110.

Expression

Each ORF cloned into the expression vector may then be transformed into the strain suitable for expression of the recombinant protein product. 1 μ l of each construct was used to transform 30 μ l of *E.coli* BL21 (pGEX vector), *E.coli* TOP 10 (pTRC vector) or *E.coli* BL21-DE3 (pET vector), as described above. In the case of the pGEX-His vector, the same *E.coli* strain (W3110) was used for initial cloning and expression. Single recombinant colonies were inoculated into 2ml LB+Amp (100 μ g/ml), incubated at 37°C overnight, then diluted 1:30 in 20 ml of LB+Amp (100 μ g/ml) in 100 ml flasks, making sure that the OD₆₀₀ ranged between 0.1 and 0.15. The flasks were incubated at 30°C into gyratory water bath shakers until OD indicated exponential growth suitable for induction of expression (0.4-0.8 OD for

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pET and pTRC vectors; 0.8-1 OD for pGEX and pGEX-His vectors). For the pET, pTRC and pGEX-His vectors, the protein expression was induced by addition of 1mM IPTG, whereas in the case of pGEX system the final concentration of IPTG was 0.2 mM. After 3 hours incubation at 30°C, the final concentration of the sample was checked by OD. In order to check expression, 1ml of each sample was removed, centrifuged in a microfuge, the pellet resuspended in PBS, and analysed by 12% SDS-PAGE with Coomassie Blue staining. The whole sample was centrifuged at 6000g and the pellet resuspended in PBS for further use.

GST-fusion proteins large-scale purification.

A single colony was grown overnight at 37°C on LB+Amp agar plate. The bacteria were inoculated into 20 ml of LB+Amp liquid culture in a water bath shaker and grown overnight. Bacteria were diluted 1:30 into 600 ml of fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD₅₅₀ 0.8-1. Protein expression was induced with 0.2mM IPTG followed by three hours incubation. The culture was centrifuged at 8000 rpm at 4°C. The supernatant was discarded and the bacterial pellet was resuspended in 7.5 ml cold PBS. The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed two times and centrifuged again. The supernatant was collected and mixed with 150µl Glutathione-Sepharose 4B resin (Pharmacia) (previously washed with PBS) and incubated at room temperature for 30 minutes. The sample was centrifuged at 700g for 5 minutes at 4°C. The resin was washed twice with 10 ml cold PBS for 10 minutes, resuspended in 1ml cold PBS, and loaded on a disposable column. The resin was washed twice with 2ml cold PBS until the flow-through reached OD₂₈₀ of 0.02-0.06. The GST-fusion protein was eluted by addition of 700µl cold Glutathione elution buffer 10mM reduced glutathione, 50mM Tris-HCl) and fractions collected until the OD₂₈₀ was 0.1. 21µl of each fraction were loaded on a 12% SDS gel using either Biorad SDS-PAGE Molecular weight standard broad range (M1) (200, 116.25, 97.4, 66.2, 45, 31, 21.5, 14.4, 6.5 kDa) or Amersham Rainbow Marker (M") (220, 66, 46, 30, 21.5, 14.3 kDa) as standards. As the MW of GST is 26kDa, this value must be added to the MW of each GST-fusion protein.

His-fusion soluble proteins large-scale purification.

A single colony was grown overnight at 37°C on a LB + Amp agar plate. The bacteria were inoculated into 20ml of LB+Amp liquid culture and incubated overnight in a water bath shaker. Bacteria were diluted 1:30 into 600ml fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD₅₅₀ 0.6-0.8. Protein expression was induced by addition of 1 mM IPTG and the culture further incubated for three hours. The culture was centrifuged at 8000 rpm at 4°C, the supernatant was discarded and the bacterial pellet was resuspended in 7.5ml cold 10mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 10 mM imidazole, pH 8). The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed two times and centrifuged again. The supernatant was collected and mixed with 150µl Ni²⁺-resin (Pharmacia) (previously washed with 10mM imidazole buffer) and incubated at room temperature with gentle agitation for 30 minutes. The sample was centrifuged at 700g for 5 minutes at 4°C. The resin was washed twice with 10 ml cold 10mM imidazole buffer for 10 minutes, resuspended in 1ml cold 10mM imidazole buffer and loaded on a disposable column. The resin was washed at 4°C with 2ml cold 10mM imidazole buffer until the flow-through reached the O.D₂₈₀ of 0.02-0.06. The resin was washed with 2ml cold 20mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 20 mM imidazole, pH 8) until the flow-through reached the O.D₂₈₀ of 0.02-0.06. The His-fusion protein was eluted by addition of 700µl cold 250mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 250 mM imidazole, pH 8) and fractions collected until the O.D₂₈₀ was 0.1. 21µl of each fraction were loaded on a 12% SDS gel.

His-fusion insoluble proteins large-scale purification.

A single colony was grown overnight at 37 °C on a LB + Amp agar plate. The bacteria were inoculated into 20 ml of LB+Amp liquid culture in a water bath shaker and grown overnight. Bacteria were diluted 1:30 into 600ml fresh medium and let to grow at the optimal temperature (37°C) to O.D₅₅₀ 0.6-0.8. Protein expression was induced by addition of 1 mM IPTG and the culture further incubated for three hours. The culture was centrifuged at 8000rpm at 4°C. The supernatant was discarded and the bacterial pellet was resuspended in 7.5 ml buffer B (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 8.8). The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen

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and thawed twice and centrifuged again. The supernatant was stored at -20°C, while the pellets were resuspended in 2 ml guanidine buffer (6M guanidine hydrochloride, 100mM phosphate buffer, 10 mM Tris-HCl, pH 7.5) and treated in a homogenizer for 10 cycles. The product was centrifuged at 13000 rpm for 40 minutes. The supernatant was mixed with 150µl Ni²⁺-resin (Pharmacia) (previously washed with buffer B) and incubated at room temperature with gentle agitation for 30 minutes. The sample was centrifuged at 700 g for 5 minutes at 4°C. The resin was washed twice with 10 ml buffer B for 10 minutes, resuspended in 1ml buffer B, and loaded on a disposable column. The resin was washed at room temperature with 2ml buffer B until the flow-through reached the OD₂₈₀ of 0.02-0.06. The resin was washed with 2ml buffer C (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 6.3) until the flow-through reached the O.D₂₈₀ of 0.02-0.06. The His-fusion protein was eluted by addition of 700µl elution buffer (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 4.5) and fractions collected until the OD₂₈₀ was 0.1. 21µl of each fraction were loaded on a 12% SDS gel.

His-fusion proteins renaturation

10% glycerol was added to the denatured proteins. The proteins were then diluted to 20µg/ml using dialysis buffer I (10% glycerol, 0.5M arginine, 50mM phosphate buffer, 5mM reduced glutathione, 0.5mM oxidised glutathione, 2M urea, pH 8.8) and dialysed against the same buffer at 4°C for 12-14 hours. The protein was further dialysed against dialysis buffer II (10% glycerol, 0.5M arginine, 50mM phosphate buffer, 5mM reduced glutathione, 0.5mM oxidised glutathione, pH 8.8) for 12-14 hours at 4°C. Protein concentration was evaluated using the formula:

$$\text{Protein (mg/ml)} = (1.55 \times \text{OD}_{280}) - (0.76 \times \text{OD}_{260})$$

Mice immunisations

20µg of each purified protein were used to immunise mice intraperitoneally. In the case of some ORFs, Balb-C mice were immunised with Al(OH)₃ as adjuvant on days 1, 21 and 42, and immune response was monitored in samples taken on day 56. For other ORFs, CD1 mice could be immunised using the same protocol. For other ORFs, CD1 mice could be immunised using Freund's adjuvant, and the same immunisation protocol was used, except that the immune response was measured on day 42, rather than 56. Similarly, for still other

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ORFs, CD1 mice could be immunised with Freund's adjuvant, but the immune response was measured on day 49.

ELISA assay (sera analysis)

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 7ml of Mueller-Hinton Broth (Difco) containing 0.25% Glucose. Bacterial growth was monitored every 30 minutes by following OD₆₂₀. The bacteria were let to grow until the OD reached the value of 0.3-0.4. The culture was centrifuged for 10 minutes at 10000 rpm. The supernatant was discarded and bacteria were washed once with PBS, resuspended in PBS containing 0.025% formaldehyde, and incubated for 2 hours at room temperature and then overnight at 4°C with stirring. 100µl bacterial cells were added to each well of a 96 well Greiner plate and incubated overnight at 4°C. The wells were then washed three times with PBT washing buffer (0.1% Tween-20 in PBS). 200 µl of saturation buffer (2.7% Polyvinylpyrrolidone 10 in water) was added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 200 µl of diluted sera (Dilution buffer: 1% BSA, 0.1% Tween-20, 0.1% NaN₃ in PBS) were added to each well and the plates incubated for 90 minutes at 37°C. Wells were washed three times with PBT. 100 µl of HRP-conjugated rabbit anti-mouse (Dako) serum diluted 1:2000 in dilution buffer were added to each well and the plates were incubated for 90 minutes at 37°C. Wells were washed three times with PBT buffer. 100 µl of substrate buffer for HRP (25 ml of citrate buffer pH5, 10 mg of O-phenildiamine and 10 µl of H₂O) were added to each well and the plates were left at room temperature for 20 minutes. 100 µl H₂SO₄ was added to each well and OD₄₉₀ was followed. The ELISA was considered positive when OD490 was 2.5 times the respective pre-immune sera.

FACScan bacteria Binding Assay procedure.

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 4 tubes containing 8ml each Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following

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OD₆₂₀. The bacteria were let to grow until the OD reached the value of 0.35-0.5. The culture was centrifuged for 10 minutes at 4000 rpm. The supernatant was discarded and the pellet was resuspended in blocking buffer (1% BSA, 0.4% NaN₃) and centrifuged for 5 minutes at 4000 rpm. Cells were resuspended in blocking buffer to reach OD₆₂₀ of 0.07. 100µl bacterial cells were added to each well of a Costar 96 well plate. 100µl of diluted (1:200) sera (in blocking buffer) were added to each well and plates incubated for 2 hours at 4°C. Cells were centrifuged for 5 minutes at 4000 rpm, the supernatant aspirated and cells washed by addition of 200µl/well of blocking buffer in each well. 100µl of R-Phicoerytrin conjugated F(ab)₂ goat anti-mouse, diluted 1:100, was added to each well and plates incubated for 1 hour at 4°C. Cells were spun down by centrifugation at 4000rpm for 5 minutes and washed by addition of 200µl/well of blocking buffer. The supernatant was aspirated and cells resuspended in 200µl/well of PBS, 0.25% formaldehyde. Samples were transferred to FACScan tubes and read. The condition for FACScan setting were: FL1 on, FL2 and FL3 off; FSC-H Threshold:92; FSC PMT Voltage: E 02; SSC PMT: 474; Amp. Gains 7.1; FL-2 PMT: 539. Compensation values: 0.

OMV preparations

Bacteria were grown overnight on 5 GC plates, harvested with a loop and resuspended in 10 ml 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30 minutes and the bacteria disrupted by sonication for 10' on ice (50% duty cycle, 50% output). Unbroken cells were removed by centrifugation at 5000g for 10 minutes and the total cell envelope fraction recovered by centrifugation at 50000g at 4°C for 75 minutes. To extract cytoplasmic membrane proteins from the crude outer membranes, the whole fraction was resuspended in 2% sarkosyl (Sigma) and incubated at room temperature for 20 minutes. The suspension was centrifuged at 10000g for 10 minutes to remove aggregates, and the supernatant further ultracentrifuged at 50000g for 75 minutes to pellet the outer membranes. The outer membranes were resuspended in 10mM Tris-HCl, pH8 and the protein concentration measured by the Bio-Rad Protein assay, using BSA as a standard.

Whole Extracts preparation

Bacteria were grown overnight on a GC plate, harvested with a loop and resuspended in 1ml of 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30' minutes.

Western blotting

Purified proteins (500ng/lane), outer membrane vesicles (5 µg) and total cell extracts (25µg) derived from MenB strain 2996 were loaded on 15% SDS-PAGE and transferred to a nitrocellulose membrane. The transfer was performed for 2 hours at 150mA at 4°C, in transferring buffer (0.3 % Tris base, 1.44 % glycine, 20% methanol). The membrane was saturated by overnight incubation at 4°C in saturation buffer (10% skimmed milk, 0.1% Triton X100 in PBS). The membrane was washed twice with washing buffer (3% skimmed milk, 0.1% Triton X100 in PBS) and incubated for 2 hours at 37°C with 1:200 mice sera diluted in washing buffer. The membrane was washed twice and incubated for 90 minutes with a 1:2000 dilution of horseradish peroxidase labeled anti-mouse Ig. The membrane was washed twice with 0.1% Triton X100 in PBS and developed with the Opti-4CN Substrate Kit (Bio-Rad). The reaction was stopped by adding water.

Bactericidal assay

MC58 strain was grown overnight at 37°C on chocolate agar plates. 5-7 colonies were collected and used to inoculate 7ml Mueller-Hinton broth. The suspension was incubated at 37°C on a nutator and let to grow until OD₆₂₀ was in between 0.5-0.8. The culture was aliquoted into sterile 1.5ml Eppendorf tubes and centrifuged for 20 minutes at maximum speed in a microfuge. The pellet was washed once in Gey's buffer (Gibco) and resuspended in the same buffer to an OD₆₂₀ of 0.5, diluted 1:20000 in Gey's buffer and stored at 25°C.

50µl of Gey's buffer/1% BSA was added to each well of a 96-well tissue culture plate. 25µl of diluted (1:100) mice sera (dilution buffer: Gey's buffer/0.2% BSA) were added to each well and the plate incubated at 4°C. 25µl of the previously described bacterial suspension were added to each well. 25µl of either heat-inactivated (56°C waterbath for 30 minutes) or normal baby rabbit complement were added to each well. Immediately after the addition of the baby rabbit complement, 22µl of each sample/well were plated on Mueller-

- 69 -

Hinton agar plates (time 0). The 96-well plate was incubated for 1 hour at 37°C with rotation and then 22µl of each sample/well were plated on Mueller-Hinton agar plates (time 1). After overnight incubation the colonies corresponding to time 0 and time 1h were counted.

The following DNA and amino acid sequences are identified by titles of the following form: [g, m, or a] [#].[seq or pep], where "g" means a sequence from *N. gonorrhoeae*, "m" means a sequence from *N. meningitidis B*, and "a" means a sequence from *N. meningitidis A*; "#" means the number of the sequence; "seq" means a DNA sequence, and "pep" means an amino acid sequence. For example, "g001.seq" refers to an *N. gonorrhoeae* DNA sequence, number 1. The presence of the suffix "-1" or "-2" to these sequences indicates an additional sequence found for the same ORF. Further, open reading frames are identified as ORF #, where "#" means the number of the ORF, corresponding to the number of the sequence which encodes the ORF, and the ORF designations may be suffixed with ".ng" or ".a", indicating that the ORF corresponds to a *N. gonorrhoeae* sequence or a *N. meningitidis A* sequence, respectively. Computer analysis was performed for the comparisons that follow between "g", "m", and "a" peptide sequences; and therein the "pep" suffix is implied where not expressly stated.

EXAMPLE 1

The following ORFs were predicted from the contig sequences and/or the full length sequences using the methods herein described.

Localization of the ORFs

ORF: contig:

279 gnm4.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 2>:
m279.seq

```

1   ATAACGCGGA TTTGCGGCTG CTTGATTTCA ACGGTTTTCA GGGCTTCGGC
51  AAGTTTGTCT GCGGCGGGTT TCATCAGGCT GCAATGGGAA GGTACGGACA
101 CGGGCAGCGG CAGGCGCGGT TTGCACCGG CTTCTTTGGC GGCAGCCATG
151 GCGCGTCCGA CGGCGGCGGC GTTGCTTGCA ATCAGGATT GTCCGGGTGA
201 GTTGAAGTTG ACGGCTTCGA CCACTTCGCT TTGGGCGGCT TCGGCACAAA
251 TGGCTTTAAC CTGCTCATCT TCCAAGCCGA GAATCGCCGC CATTGCGCCC
301 ACGCCTTGCG GTACGGCGGA CTGCATCAGT TCGGCGCGCA GGCGCACGAG
351 TTTGACCGCG TCGGCAAAAT TCAATGCGCC GGCGGCAACG AGTGCGGTGT
401 ATTCGCCGAG GCTGTGTCCG GCAACGGCGG CAGGCGTTTT GCCGCCCGCT
451 TCTAAATAG

```

1 ITRICGLIS TVFRASASLS AAGFIRLOWE GTDTGSGRAR LAPASLAAAM
51 ARPTAAALPA ITICPGELKL TASTTSLWAA SAQMALTCSS SKPRIAAIAP
101 TPCGTADCIS SARRRTSLTA SAKFNAPAAAT SAVYSPRLCP ATAAGVLPPA
151 SK*

The following partial DNA sequence was identified in *N.gonorrhoeae* <SEQ ID 4>:

seq	atgacgcgga	tttgcggtg	cttgatttca	acggttttga	gtgtttcggc
51	aagtttgtcg	gcggcggtt	tcacaggct	gcaatgggaa	ggaacggata
101	ccggcagcgg	cagggcgct	ttggctccgg	cttctttggc	ggcagccatg
151	gtgcgtccga	cggcgcggc	gttgctgcga	atcacgactt	gtccgggcga
201	gttgaagtgt	acggcttcga	ccacttcgcc	ctgtgcggat	tcggcacaaa
251	tctgctgac	ctgttcatt	tccaaaccca	aaatggccgc	cattgcgcct
301	acgccttgca	gtacggcgga	ctgcatcagt	tcggcgcgca	ggcggaacgag
351	tttagcggca	tcggcaaaa	ccaatgcttc	ggcggcgaca	agcgcggtgt
401	attcgcgcag	gctgtgtccg	gcaacggcgg	caggcggttt	gccgccact
451	tccaaatag				

This corresponds to the amino acid sequence <SEQ ID 5; ORF 279.ng>:

```

1  MTRICGCLIS TVLSVSASLS AAGFIRLOWE GTDTGSGRAR LAPASLAAAM
51  VRPTAAALPA ITTCPGELKL TASTTSPCAD SAQICLTSS SKPKMAAIAP
101 TPCGTADCIS SARRRTSLTA SAKSNSAAT SAVYSPRLCP ATAAGVLPPT
151 SK*

```

```

      10          20          30          40          50          60
m279.pep  ITRICGCLISTVFRASASLSAAGFIRLQWEGTDTGSGRRARLAPASLAAAMARPTAAALPA
          :|||||||||: :|||||||||||||||||||||||||||||||||:|||||||
g279      MTRICGCLISTVLSVSASLSAAGFIRLQWEGTDTGSGRRARLAPASLAAAMVRPTAAALPA
          10          20          30          40          50          60

      70          80          90          100         110         120
m279.pep  ITICPGELKLTASTTSLWAASAQMALTCSSSKPRIAAIAPTCGTADCISSARRRTSLTA
          || ||||||||||||| |||: |||||||: :||| |||||||||||||||||
g279      ITTCPGELKLTASTTSPCADSAQICLTCSKPKMAAIAPTCGTADCISSARRRTSLTA
          70          80          90          100         110         120

      130         140         150
m279.pep  SAKFNAPAATSAVYSPRLCPATAAGVLPSPASKX
          ||| || ||||||||||||| |||: |||
g279      SAKSNASAATSAVYSPRLCPATAAGVLPPTSKX
          130         140         150

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 6>:

seq	1	ATGACNCNGA	TTTGGCGGCTG	CTTGATTTC	ACGGTTTNNNA	GGGCTTCGGC
51		GAGTTTGTCG	GCGGCGGGTT	TCATGAGGCT	GCAATGGGAA	GGTACNGACA
101		CNGGCAGCGG	CAGGGCGCGT	TTGGCGCCGG	CTTCTTTGGC	GGCAAGCATA
151		GC GCCTCGA	CGGCGGCGGC	ATTGCTGCA	ATCACGACTT	GTCCGGGCGA
201		GTTGAAGTTG	ACGGCTTCAA	CCACTTCATC	CTGTGCGGAT	TCCGGCGCAA
251		TTTGTTTTAC	CTGTTCTCAT	TCCAAGCCGA	GAATCGCGCG	CATTGCGCCC
301		ACGCTTTCGC	TACGGCGCGA	CTGCATCAGT	TCGGCGCGCA	NGCGCACAG
351		TTTGACCGCG	TCGGCAAAT	CCAATGCGCC	GGCGGCAACN	AGTGC GG TGT

401 ATTCGCCGAN GCTGTGTCCG GCAACGGCGG CAGGCGTTTT GCCGCCCCGT
451 TCCGAATAG

a279.pep

1	MTXICGCLIS	TVXRASASLS	AAGFMRLQWE	GTDTGSGRAR	LAPASLAASI
51	ARSTAAALPA	ITTCPGELKL	TASTTSSCAD	SAQICFTCSS	SKPRIAAAIAP
101	TPCGTADCIS	SARXRTSLTA	SAKSNAPAAT	SAVYSXPXLC	ATAAGVLPPA
151	SE*				

```

              10          20          30          40          50          60
m279.pep      ITRICGLISTVFRASASLSAAGFIRLQWEGTDTGSGRRARLAPASLAAMARPTAAALPA
               :| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
a279           MTXICGLISTVXRASASLSAAGFMRLQWEGTDTGSGRRARLAPASLAASIARSTAAALPA
               10          20          30          40          50          60

              70          80          90          100         110         120
m279.pep      ITICPGELKLTASTTSLWAAASQMALTCSSSKPRIAAIAPTPCGTADCISSARRRTSLTA
               || ||||| ||||| ||||| :||| ||||| ||||| ||||| ||||| |||||
a279           ITTCPGELKLTASTTSSCADSAQICFTCSSSKPRIAAIAPTPCGTADCISSARXRTSLTA
               70          80          90          100         110         120

              130         140         150
m279.pep      SAKFNAPAATSAVYSPRLCPATAAGVLPPASKX
               ||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
a279           SAKSNAPAATSAVYSPXLCPATAAGVLPPASEX
               130         140         150

```

```
m519.seq (partial)
1 ..TCCGTTATCG GGCATATGGA GTTGGACAAA ACGTTTGAAG AACGCGACGA
51 AATCAACAGT ACTGTTGTTG CGGCTTTGGA CGAGGCGGCC GGgGCTTgGG
101 GTGTGAAGGT TTTGCGTTAT GAGATTAAAG ACTTGGTTCC GCCGAAGAA
151 ATCCTTCGCT CAATGCAGGC GCAAATTACT GCCGAACGCG AAAAACGCGC
201 CCGTATCGCC GATCCGAAG CTCGTAAAT CGAACAAATC AACCTTGCCA
251 GTGGTCAGCG CGAAGCCGAA ATCCAACAAT CCGAAGGCGA GGCTCAGGCT
301 GCGGTCAATG CGTCAAATGC CGAGAAAATC GCCCGCATCA ACCGCGCCAA
351 AGGTGAAGCG GAATCCTTGC GCCTTGTGTC CGAAGCCAAT GCCGAAGCCA
401 TCCGTCAAAT TGCCGCCGCC CTTCAAACCC AAGGCGGTGC GGATGCGGTC
451 AATCTGAAGA TTGCGGAACA ATACGTCGCT GC GTTCAACA ATCTTGCCAA
501 AGAAAGCAAT ACGCTGATTA TGCCCGCCAA TGTGTCCGAC ATCGGCGACC
551 TGATTCTTGC CGGTATGAAA ATTATCGACA CGACGAAAAC CGCCAAaTA
```

```
m519.pep      (partial)
1      ..SVIGRMELDK TFEERDEINS TVVAALDEAA GAWGVKVLRY EIKDLVPPQE
51     ILRSMQAQIT AEREKRARIA ESEGRKIEQI NLASGQREAE IQQSEGEAAQ
101    AVNNSAAEKI ARINRKAGEA ESLRLVAEAN AEAIRQIAAA LQTQGGADAV
151    NLKIAEQYVA AFNNLAKESN TLIMPANVAD IGSLSAGMK IIDSSKTA*
```

g519.seq
1 atggaatttt tcattatctt gttggcagcc gtcgccgttt tcggcttcaa
51 atcctttgtc gtcacccccc agcaggaagt ccacgttgtc gaaaggctcg

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```

101  ggcgtttcca tcgcgccttg acggccggtt tgaatatattt gattcccttt
151  atcgaccgcg tcgcctaccg ccattcgctg aaagaaatcc ctttagacgt
201  acccagccag gtctgcatca cgcgcgataa tacgcaattg actgttgacg
251  gcatcatcta tttccaagta accgatccca aactcgcctc atacggttcg
301  agcaactaca ttatggcaat taccagctt gcccaaacga cgctgcgttc
351  cgttatcggg cgtatggagt tggacaaaac gtttgaagaa cgcgcgaaaa
401  tcaacagtac cgtcgtctcc gccctcgatg aagccgcgcg ggcttggggg
451  gtgaaagtcc tccgttacga aatcaaggat ttggttccgc cgcaagaaat
501  ccttcgcgca atgcaggcac aaattaccgc cgaacgcgaa aaacgcgccc
551  gtattgccga atccgaaggc cgtaaaaatcg aacaaatcaa ccttgccagt
601  ggtcagcgtg aagccgaaat ccaacaatcc gaaggcgagg ctcaggctgc
651  ggtcaatgcg tccaatgccg agaaaatcgc ccgcatcaac cgcgccaaag
701  gcgaagcgga atccctgcgc cttgttgccg aagccaatgc cgaagccaac
751  cgtcaaattg ccgccgccct tcaaacccaa agcggggcgcg atgcggtcaa
801  tctgaagatt gcgggacaat acgttaccgc gttcaaaaat cttgccaaag
851  aagacaatac gcggattaag cccgcccaagg ttgccgaaat cgggaaccct
901  aattttcggc ggcattgaaa attttcgccg gaagcaaaaa cggcctaata
951  a

```

This corresponds to the amino acid sequence <SEQ ID 11; ORF 519.ng>:

```

g519.pep
1  MEFFIILLAA VAVFGFKSFV VIPQEVHV V ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHSL KEIPLDVPSQ VCITRDNTQL TVDGIIFYQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRA MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAEIQQS EGEAQAAVNA SNAEKIARIN RAKGEAESLR LVAEANA EAN
251 RQIAAALQTO SGADAVNLKI AGQYVTAFAKN LAKEDNTRIK PAKVAEIGNP
301 NFRRHEKFSP EAKTAK*

```

ORF 519 shows 87.5% identity over a 200 aa overlap with a predicted ORF (ORF 519.ng) from *N. gonorrhoeae*:

m519/g519

m519.pep					10	20	30
					SVIGRMELDKTFEERDEINSTVVAALDEAA		
g519							
	90	100	110	120	130	140	
m519.pep		40	50	60	70	80	90
		GAWGVKVLRYEIKDLVPPQEILRS	MQAQITAEREKRARIAESEGRKIEQINLASGQREAE				
g519							
	150	160	170	180	190	200	
m519.pep		100	110	120	130	140	150
		IQQSEGEAQAAVNASNAEKIARINRAKGEAESLRLVAEANA EAI RQIAAALQTQGGADAV					
g519							
	210	220	230	240	250	260	
m519.pep		160	170	180	190	200	
		NLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL- ISAGMKIIDSSKTAK					
g519							
	270	280	290	300	310		
		NLKIAGQYVTAFAKNLAKEDNTRIKPAKVAEIGNPNFRRHEKFSP EAKTAK					

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 12>:

a519.seq

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```

1  ATGAATTTT TCATTATCTT GCTGGCAGCC GTCGTTGTTT TCGGCTTCAA
51  ATCCTTTGTT GTCATCCAC AGCAGGAAGT CCACGTTGTC GAAAGGCTCG
101 GGCCTTTCCA TCGCGCCCTG ACGGCCGCTT TGAATATTTT GATTCCCTTT
151 ATCGACCGCG TCGCCTACCG CCATTCGCTG AAAGAAATCC CTTTAGACGT
201 ACCAGCCAG GTCTGCATCA CGCGCGACAA TACGCAGCTG ACTGTTGACG
251 GTATCATCTA TTTCCAAGTA ACCGACCCCA AACTCGCCTC ATACGGTTCTG
301 AGCAACTACA TTATGGCGAT TACCCAGCTT GCCCAAACGA CGCTGCGTTC
351 CGTTATCGGG CGTATGGAAT TGGACAAAAC GTTGAAGAA CGCGACGAAA
401 TCAACAGCAC CGTCGTCTCC GCCCTCGATG AAGCCGCCGG AGCTTGGGGT
451 GTGAAGGTTT TGCCTTATGA GATTAAAGAC TTGGTTCCGC CGCAAGAAAT
501 CCTTCGCTCA ATGCAGGCGC AAATTACTGC TGAACGCGAA AAACGCGCCC
551 GTATCGCCGA ATCCGAAGGT CGTAAATCG AACAAATCAA CCTTGCCAGT
601 GGTGAGCGCG AAGCCGAAAT CCAACAATCC GAAGCGGAGG CTCAGGCTGC
651 GGTCATCGCG TCAAATGCCG AGAAAATCGC CCGCATCAAC CGCGCCAAAG
701 GTGAAGCGGA ATCCTTGCGC CTTGTTGCCG AAGCCAATGC CGAAGCCATC
751 CGTCAAATTG CGCGCGCCCT TCAAACCCAA GCGCGTGCGG ATGCGGTCAA
801 TCTGAAGATT GCGGAACAAT ACGTCGCCGC GTTCAACAAT CTTGCCAAAG
851 AAAGCAATAC GCTGATTATG CCCGCCAATG TTGCCGACAT CGGCAGCCTG
901 ATTTCTGCCG GTATGAAAAT TATCGACAGC AGCAAACCG CCAAATAA

```

This corresponds to the amino acid sequence <SEQ ID 13; ORF 519.a>:

```

a519.pep
1  MEFFIILLAA VVVFQFKSFV VIPQOEHVHV ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHSI KEIPLDVPSQ VCITRDNTQL TVDGIIFYQV TDPKLSYGS
101 SNYIMAITQL AQTTLRVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRS MQAQITAERE KRARIAESEGRKIEQINLAS
201 GQREAEIQQS EGQAQAVNA SNAEKIARIN RAKGEAESLR LVAEANAELAI
251 RQIAAALQTO GGADAVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

m519/a519  ORFs 519 and 519.a showed a 99.5% identity in 199 aa overlap

m519.pep                                     10      20      30
SVIGRMELDKTFEERDEINSTVVAALDEAA
a519      YFQVTDPKLASYGSSNYIMAITQLAQTTLRVIGRMELDKTFEERDEINSTVVSALDEAA
          90      100      110      120      130      140

m519.pep          40      50      60      70      80      90
GAWGVKVLRYEIKDLVPPQEILRSMAQITAEREKRARIAESEGRKIEQINLASGQREAE
a519      GAWGVKVLRYEIKDLVPPQEILRSMAQITAEREKRARIAESEGRKIEQINLASGQREAE
          150      160      170      180      190      200

m519.pep          100      110      120      130      140      150
IQQSEGEAQAVNASNAEKIARINRAKGEAESLRLVAEANAELAIRQIAAALQTOGGADAV
a519      IQQSEGEAQAVNASNAEKIARINRAKGEAESLRLVAEANAELAIRQIAAALQTOGGADAV
          210      220      230      240      250      260

m519.pep          160      170      180      190      200
NLKIAEQYVAAFNNLAKESNTLIMPANVADIGSLISAGMKIIDSSKTAKX
a519      NLKIAEQYVAAFNNLAKESNTLIMPANVADIGSLISAGMKIIDSSKTAKX
          270      280      290      300      310

```

Further work revealed the following DNA sequence identified in *N. meningitidis* <SEQ ID 14>:

m519-1.seq

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```

1  ATGGAATTTT TCATTATCTT GTTGGTAGCC GTCGCCGTTT TCGGTTTCAA
51  ATCCTTTGTT GTCATCCAC AACAGGAAGT CCACGTTGTC GAAAGGCTGG
101 GGCCTTTCCA TCGCGCCTG ACGGCGGTT TGAATATTTT GATTCCCTTT
151 ATCGACCGCG TCGCCTACCG CCATTGCTG AAAGAAATCC CTTTAGACGT
201 ACCCAGCCAG GTCTGCATCA CGCGGACAA TACGCAGCTG ACTGTTGACG
251 GCATCATCTA TTTCCAAGTA ACCGACCCA AACTCGCCTC ATACGGTTCG
301 AGCAACTACA TTATGGCGAT TACCCAGCTT GCCCAAACGA CGCTGCGTTC
351 CGTTATCGGG CGTATGGAGT TGGACAAAAC GTTTGAAGAA CGCGACGAAA
401 TCAACAGTAC TGTTGTTGCG GCTTTGGACG AGGCGGCCGG GGCTTGGGGT
451 GTGAAGGTTT TCGGTTATGA GATTAAAGAC TTGGTTCCGC CGCAAGAAAT
501 CCTTCGCTCA ATGCAGGCGC AAATTACTGC CGAACGCGAA AAACGCGCCC
551 GTATCGCCGA ATCCGAAGGT CGTAAAATCG AACAAATCAA CTTGCCAGT
601 GGTCAGCGCG AAGCCGAAAT CCAACAATCC GAAGGCGAGG CTCAGGCTGC
651 GGTCAATGCG TCAAATGCCG AGAAAATCGC CCGCATCAAC CGCGCCAAAG
701 GTGAAGCGGA ATCCTTGCGC CTTGTTGCCG AAGCCAATGC CGAAGCCATC
751 CGTCAAATTG CCGCCGCCCT TCAAACCCAA GGCGGTGCGG ATGCGGTCAA
801 TCTGAAGATT GCGGAACAAT ACGTCGCTGC GTTCAACAAT CTTGCCAAAG
851 AAAGCAATAC GCTGATTATG CCGCCAATG TTGCCGACAT CGGCAGCCTG
901 ATTTCTGCCG GTATGAAAAT TATCGACAGC AGCAAACCG CCAAATAA

```

This corresponds to the amino acid sequence <SEQ ID 15; ORF 519-1>:

m519-1.

```

1  MEFFIILLVA VAVFGFKSFV VIPQQEVHVV ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHSI KEIPLDVPSQ VCITRDNTQL TVDGIIFYQV TDPKLSYGS
101 SNYIMAITQL AQTTLSRVIG RMELDKTFEE RDEINSTVVA ALDEAAGAWG
151 VKVLYEIKD LVPPQEILRS MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAEIQQS EGEAQAQVNA SNAEKIARIN RAKGEAESLR LVAEANAIAI
251 RQIAAALQIQ GGADAVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

```

The following DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 16>:

g519-1.seq

```

1  ATGGAATTTT TCATTATCTT GTTGGCAGCC GTCGCCGTTT TCGGCTTCAA
51  ATCCTTTGTC GTCATCCCCC AGCAGGAAGT CCACGTTGTC GAAAGGCTCG
101 GGCCTTTCCA TCGCGCCTG ACGGCGGTT TGAATATTTT GATTCCCTTT
151 ATCGACCGCG TCGCCTACCG CCATTGCTG AAAGAAATCC CTTTAGACGT
201 ACCCAGCCAG GTCTGCATCA CGCGGATAA TACGCAATTG ACTGTTGACG
251 GCATCATCTA TTTCCAAGTA ACCGATCCCA AACTCGCCTC ATACGGTTCG
301 AGCAACTACA TTATGGCAAT TACCCAGCTT GCCCAAACGA CGCTGCGTTC
351 CGTTATCGGG CGTATGGAGT TGGACAAAAC GTTTGAAGAA CGCGACGAAA
401 TCAACAGTAC CGTCGTCTCC GCCCTCGATG AAGCCGCCGG GGCTTGGGGT
451 GTGAAGTCC TCCGTACGA AATCAAGGAT TTGGTTCCGC CGCAAGAAAT
501 CCTTCGCGCA ATGCAGGCAC AAATTACCGC CGAACGCGAA AAACGCGCCC
551 GTATTGCCGA ATCCGAAGGC CGTAAAATCG AACAAATCAA CTTGCCAGT
601 GGTCAGCGTG AAGCCGAAAT CCAACAATCC GAAGGCGAGG CTCAGGCTGC
651 GGTCAATGCG TCCAATGCCG AGAAAATCGC CCGCATCAAC CGCGCCAAAG
701 GCGAAGCGGA ATCCCTGCGC CTTGTTGCCG AAGCCAATGC CGAAGCCATC
751 CGTCAAATTG CCGCCGCCCT TCAAACCCAA GGCGGGGCGG ATGCGGTCAA
801 TCTGAAGATT GCGGAACAAT ACGTAGCCGC GTTCAACAAT CTTGCCAAAG
851 AAAGCAATAC GCTGATTATG CCGCCAATG TTGCCGACAT CGGCAGCCTG
901 ATTTCTGCCG GCATGAAAAT TATCGACAGC AGCAAACCG CCAAATAA

```

This corresponds to the amino acid sequence <SEQ ID 17; ORF 519-1.ng>:

g519-1.pep

```

1  MEFFIILLAA VAVFGFKSFV VIPQQEVHVV ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHSI KEIPLDVPSQ VCITRDNTQL TVDGIIFYQV TDPKLSYGS
101 SNYIMAITQL AQTTLSRVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLYEIKD LVPPQEILRA MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAEIQQS EGEAQAQVNA SNAEKIARIN RAKGEAESLR LVAEANAIAI
251 RQIAAALQIQ GGADAVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*

```

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m519-1/g519-1 ORFs 519-1 and 519-1.ng showed a 99.0% identity in 315 aa overlap

	10	20	30	40	50	60
g519-1.pep	MEFFIILLAAVAVFGFKSFVVIPQQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHSL					
	:					
m519-1	MEFFIILLVAVAVFGFKSFVVIPQQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHSL					
	10	20	30	40	50	60
	70	80	90	100	110	120
g519-1.pep	KEIPLDVPSQVCITRDNTQLTVDGIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG					
m519-1	KEIPLDVPSQVCITRDNTQLTVDGIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG					
	70	80	90	100	110	120
	130	140	150	160	170	180
g519-1.pep	RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVPPQEILRAMQAQITAERE					
	:					
m519-1	RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE					
	130	140	150	160	170	180
	190	200	210	220	230	240
g519-1.pep	KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR					
m519-1	KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR					
	190	200	210	220	230	240
	250	260	270	280	290	300
g519-1.pep	LVAEANAEAIRQIAAALQTGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL					
m519-1	LVAEANAEAIRQIAAALQTGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL					
	250	260	270	280	290	300
	310					
g519-1.pep	ISAGMKIIDSSKTAKX					
m519-1	ISAGMKIIDSSKTAKX					
	310					

The following DNA sequence was identified in *N. meningitidis* <SEQ ID 18>:

a519-1.seq

1	ATGGAATTTT	TCATTATCTT	GCTGGCAGCC	GTCGTTGTTT	TCGGCTTCAA
51	ATCCTTTGTT	GTCATCCAC	AGCAGGAAGT	CCACGTTGTC	GAAAGGCTCG
101	GGCGTTTCCA	TCGCGCCCTG	ACGGCCGGTT	TGAATATTTT	GATTCCCTTT
151	ATCGACCGCG	TCGCCTACCG	CCATTTCGCTG	AAAGAAATCC	CTTTAGACGT
201	ACCCAGCCAG	GTCTGCATCA	CGCGCGACAA	TACGCAGCTG	ACTGTTGACG
251	GTATCATCTA	TTTCCAAGTA	ACCGACCCCA	AACTCGCCTC	ATACGGTTCG
301	AGCAACTACA	TTATGGCGAT	TACCCAGCTT	GCCCCAACGA	CGCTGCGTTC
351	CGTTATCGGG	CGTATGGAAT	TGGACAAAAC	GTTTGAAGAA	CGCGACGAAA
401	TCAACAGCAC	CGTCGTCTCC	GCCCTCGATG	AAGCCGCCGG	AGCTTGGGGT
451	GTGAAGGTTT	TGCGTTATGA	GATTAAAGAC	TTGGTTCCGC	CGCAAGAAAT
501	CCTTCGCTCA	ATGCAGGCGC	AAATTACTGC	TGAACGCGAA	AAACGCGCCC
551	GTATCGCCGA	ATCCGAAGGT	CGTAAAATCG	AACAAATCAA	CCTTGCCAGT
601	GGTCAGCGCG	AAGCGGAAAT	CCAACAATCC	GAAGCGGAGG	CTCAGGCTGC
651	GGTCAATGCG	TCAAATGCCG	AGAAAATCGC	CCGCATCAAC	CGCGCCCAAG
701	GTGAAGCGGA	ATCCTTGCGC	CTTGTTGCCG	AAGCCAATGC	CGAAGCCATC
751	CGTCAAATTG	CCGCCGCCCT	TCAAACCCAA	GGCGGTGCCG	ATGCGGTCAA
801	TCTGAAGATT	GCGGAACAAT	ACGTCGCCGC	GTTCAACAAT	CTTGCCAAAG
851	AAAGCAATAC	GCTGATTATG	CCCGCAATG	TTGCCGACAT	CGGCAGCCTG
901	ATTTCTGCCG	GTATGAAAAT	TATCGACAGC	AGCAAAACCG	CCAAATAA

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This corresponds to the amino acid sequence <SEQ ID 19; ORF 519-1.a>:

a519-1.pep.

```

1  MEFFFIILLAA VVVFGEKSFV VIPQEVHV V ERLGRFHRAL TAGLNILIPF
51  IDRVAYRHS L KEIPLDVPSQ VCITRDNTQL TVDGIIYFQV TDPKLASYGS
101 SNYIMAITQL AQTTLRSVIG RMELDKTFEE RDEINSTVVS ALDEAAGAWG
151 VKVLRYEIKD LVPPQEILRS MQAQITAERE KRARIAESEG RKIEQINLAS
201 GQREAEIQQS EGQAQAVNA SNAEKIARIN RAKGEAESLR LVAEANA EAI
251 RQIAAALQTQ GGADAVNLKI AEQYVAAFNN LAKESNTLIM PANVADIGSL
301 ISAGMKIIDS SKTAK*
```

m519-1/a519-1 ORFs 519-1 and 519-1.a showed a 99.0% identity in 315 aa overlap

a519-1.pep	10	20	30	40	50	60
m519-1	10	20	30	40	50	60
a519-1.pep	70	80	90	100	110	120
m519-1	70	80	90	100	110	120
a519-1.pep	130	140	150	160	170	180
m519-1	130	140	150	160	170	180
a519-1.pep	190	200	210	220	230	240
m519-1	190	200	210	220	230	240
a519-1.pep	250	260	270	280	290	300
m519-1	250	260	270	280	290	300
a519-1.pep	310					
m519-1	310					

576 and 576-1 gnm22.seq

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 20>:

m576.seq.. (partial)

```

1  ..ATGCAGCAGG CAAGCTATGC GATGGGCGTG GACATCGGAC GCTCCCTGAA
51  GCAAAATGAAG GAACAGGGCG CGGAAATCGA TTTGAAAGTC TTTACCGAAG
101 CCATGCAGGC AGTGTATGAC GGCAAAGAAA TCAAATGAC CGAAGAGCAG
151 GCTCAGGAAG TCATGATGAA ATTCCTTCAG GAACAACAGG CTAAAGCCGT
201 AGAAAAACAC AAGGCGGACG CGAAGGCCAA TAAAGAAAAA GGCGAAGCCT
```

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```

251 TTCTGAAAGA AAATGCCGCC AAAGACGGCG TGAAGACCAC TGCTTCCGGC
301 CTGCAATACA AAATCACCAA ACAGGGCGAA GGCAAACAGC CGACCAAAGA
351 CGACATCGTT ACCGTGGAAT ACGAAGGCCG CCTGATTGAC GGTACGGTAT
401 TCGACAGCAG CAAAGCCAAC GGCGGCCCGG TCACCTTCCC TTTGAGCCAA
451 GTGATTCCGG GTTGGACCGA AGCGGTACAG CTTCTGAAAG AAGCGGCGCA
501 AGCCACGTTC TACATCCCGT CCAACCTTGC CTACCGCGAA CAGGGTGC GG
551 GCGACAAAAT CGGTCCGAAC GCCACTTTGG TATTTGATGT GAAACTGGTC
601 AAAATCGCGC CACCCGAAAA CGCGCCCGCC AAGCAGCCGG CTAAGTCGA
651 CATCAAAAAA GTAAATTAA

```

This corresponds to the amino acid sequence <SEQ ID 21; ORF 576>:

m576.pep.. (partial)

```

1 ..MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTEAMQAVYD GKEIKMTEEQ
51 AQEVMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG
101 LOYKITKQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPVTFPLSQ
151 VIPGWTEGVQ LLKEGGEATF YIPSNLAYRE QGAGDKIGPN ATLVFVVKLV
201 KIGAPENAPA KQPAQVDIKK VN*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 22>:

g576.seq.. (partial)

```

1 ..atggcgctgg acatcggagc ctccctgaaa caaatgaagg aacagggcgc
51 ggaaatcgat ttgaaagtct ttaccgatgc catgcaggca gtgtatgacg
101 gcaaagaaat caaaatgacc gaagagcagg cccaggaaat gatgatgaaa
151 ttctgcagg agcagcaggc taaagccgta gaaaaacaca aggcggatgc
201 gaaggccaac aaagaaaaag gcgaagcctt cctgaaggaa aatgccgccg
251 aagacggcgt gaagaccact gcttcgggtc tgcagtacaa aatcaccaaa
301 cagggtgaag gcaaacagcc gacaaaagac gacatcgta ccgtggaata
351 cgaaggccgc ctgattgacg gtaccgtatt cgacagcagc aaagccaacg
401 gcggcccggc caccttcctt ttgagccaag tgattccggg ttggaccgaa
451 ggcgtacggc ttctgaaaga aggcggcgaa gccacgttct acatcccgtc
501 caaccttgcc taccgcgaac aggtgacggg cgaaaaaatc ggtccgaacg
551 ccactttggt atttgacgtg aaactggtca aaatcggcgc acccgaaaac
601 gcgcccgcga agcagccgga tcaagtcgac atcaaaaaag taaattaa

```

This corresponds to the amino acid sequence <SEQ ID 23; ORF 576.ng>:

g576.pep.. (partial)

```

1 ..MGVDIGRSLK QMKEQGAEID LKVFTDAMQA VYDGKEIKMT EEQAQEVMMK
51 FLQEQQAKAV EKHKADAKAN KEKGEAFLKE NAAEDGVKTT ASGLQYKITK
101 QGEGKQPTKD DIVTVEYEGR LIDGTVFDSS KANGGPATFP LSQVIPGWTE
151 GVRLLKEGGE ATFYIPSNLA YREQGAGEKI GPNATLVFDV KLVKIGAPEN
201 APAKQPDQVD IKKVN*

```

Computer analysis of this amino acid sequence gave the following results:

Homology with a predicted ORF from *N. gonorrhoeae*

m576/g576 97.2% identity in 215 aa overlap

```

              10      20      30      40      50      60
m576.pep  MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTEAMQAVYD GKEIKMTEEQ AQEVMKFLQ
              |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
g576      MGVDIGRSLKQMK EQGAEIDLKV FTEAMQAVYD GKEIKMTEEQ AQEVMKFLQ
              10      20      30      40      50

              70      80      90     100     110     120
m576.pep  EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTAS GLQYKITK QGEGKQPTK DDIV
              |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
g576      EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTAS GLQYKITK QGEGKQPTK DDIV
              60      70      80      90     100     110

```

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	130	140	150	160	170	180
m576.pep	TVEYEGRLIDGTVFDSSKANGGPVTFPLSQVIPGWTEGVQLLKEGGEATFYIPSNLAYRE					
	: : : : :					
g576	TVEYEGRLIDGTVFDSSKANGGPATFPLSQVIPGWTEGVRLLEKGEATFYIPSNLAYRE					
	120	130	140	150	160	170

	190	200	210	220
m576.pep	QGAGDKIGPNATLVFDVKLVKIGAPENAPAKQPAQVDIKKVN			
	: : :			
g576	QGAGEKIGPNATLVFDVKLVKIGAPENAPAKQPDQVDIKKVN			
	180	190	200	210

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 24>:

```

a576.seq
1  ATGAACACCA TTTTCAAAAT CAGCGCACTG ACCCTTTCCG CCGCTTTGGC
51  ACTTTCCGCC TGCGGCAAAA AAGAAGCCGC CCCCGCATCT GCATCCGAAC
101 CTGCCGCCGC TTCTTCCGCG CAGGGCGACA CCTCTTCGAT CGGCAGCACG
151 ATGCAGCAGG CAAGCTATGC GATGGGCGTG GACATCGGAC GCTCCCTGAA
201 GCAAATGAAG GAACAGGGCG CGGAAATCGA TTTGAAAGTC TTTACCGAAG
251 CCATGCAGGC AGTGTATGAC GGCAGAGAAA TCAAATGAC CGAAGAGCAG
301 GCTCAGGAAG TCATGATGAA ATTCCTTCAG GAACAACAGG CTAAAGCCGT
351 AGAAAAACAC AAGGCGGACG CGAAGGCCAA TAAAGAAAAA GGCGAAGCCT
401 TTCTGAAAGA AAATGCCGCG AAAGACGGCG TGAAGACCAC TGCTTCCGGC
451 CTGCAATACA AAATCACCAA ACAGGGCGAA GGCAAACAGC CGACCAAAGA
501 CGACATCGTT ACCGTGGAAT ACGAAGGCCG CCTGATTGAC GGTACGGTAT
551 TCGACAGCAG CAAAGCCAAC GGCGGCCCGG TCACCTTCCC TTTGAGCCAA
601 GTGATTCTGG GTTGGACCGA AGGCGTACAG CTTCTGAAAG AAGGCGGCGA
651 AGCCACGTTC TACATCCCGT CCAACCTTGC CTACCGCGAA CAGGGTGCGG
701 GCGACAAAT CGGCCCGAAC GCCACTTTGG TATTTGATGT GAAACTGGTC
751 AAAATCGGCG CACCCGAAAA CGCGCCCGCC AAGCAGCCGG CTCAAGTCGA
801 CATCAAAAAA GTAAATTAA

```

This corresponds to the amino acid sequence <SEQ ID 25; ORF 576.a>:

```

a576.pep
1  MNTIFKISAL TLSAALALSA CGKKEAAPAS ASEPAAASSA QGDTSSIGST
51  MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTEAMQAVYD GKEIKMTEEQ
101 AQEVMKFLQ EQQAKAVEKH KADAKANKEK GEAFLKENAA KDGVKTTASG
151 LQYKITKQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPVTFPLSQ
201 VILGWTEGVQ LLKEGGEATF YIPSNLAYRE QGAGDKIGPN ATLVFDVKLV
251 KIGAPENAPA KQPAQVDIKK VN*

```

m576/a576 ORFs 576 and 576.a showed a 99.5% identity in 222 aa overlap

		10	20	30
m576.pep		MQQASYAMGV DIGRSLKQMK EQGAEIDLKV		
		: : :		
a576		CGKKEAAPAS ASEPA A ASSA QGDTSSIGSTMQQASYAMGV DIGRSLKQMK EQGAEIDLKV		
	30	40	50	60

	40	50	60	70	80	90
m576.pep	FTEAMQAVYD GKEIKMTEEQ AQEVMKF L Q EQQAKAVEKH KADAKANKEK GEAF L KENAA					
	: : :					
a576	FTEAMQAVYD GKEIKMTEEQ AQEVMKF L Q EQQAKAVEKH KADAKANKEK GEAF L KENAA					
	90	100	110	120	130	140

	100	110	120	130	140	150
m576.pep	KDG V KTTASGLQYKITKQEGGKQPTKDDIV TVEYEGRLIDGTVFDSSKANGGPVTFPLSQ					
	: : :					
a576	KDG V KTTASGLQYKITKQEGGKQPTKDDIV TVEYEGRLIDGTVFDSSKANGGPVTFPLSQ					
	150	160	170	180	190	200

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	160	170	180	190	200	210
m576.pep	VIPGWTEGVQLLKEGGEATFYIPSNLAYREQGAGDKIGPNATLVFDVKLVKIGAPENAPA					
a576	VILGWTEGVQLLKEGGEATFYIPSNLAYREQGAGDKIGPNATLVFDVKLVKIGAPENAPA					
	210	220	230	240	250	260
	220					
m576.pep	KQPAQVDIKKVN					
a576	KQPAQVDIKKVN					
	270					

Further work revealed the following DNA sequence identified in *N. meningitidis* <SEQ ID 26>:

m576-1.seq

1	ATGAACACCA	TTTTCAAAAT	CAGCGCACTG	ACCCTTTCCG	CCGCTTTGGC
51	ACTTTCCGCC	TGCGGCAAAA	AAGAAGCCGC	CCCCGCATCT	GCATCCGAAC
101	CTGCCGCCGC	TTCTTCCGCG	CAGGGCGACA	CCTCTTCGAT	CGGCAGCACG
151	ATGCAGCAGG	CAAGCTATGC	GATGGGCGTG	GACATCGGAC	GCTCCCTGAA
201	GCAAATGAAG	GAACAGGGCG	CGGAAATCGA	TTTGAAAGTC	TTTACCGAAG
251	CCATGCAGGC	AGTGTATGAC	GGCAAAGAAA	TCAAATGAC	CGAAGAGCAG
301	GCTCAGGAAG	TCATGATGAA	ATTCTTCAG	GAACAACAGG	CTAAAGCCGT
351	AGAAAAACAC	AAGGCGGACG	CGAAGGCCAA	TAAAGAAAAA	GGCGAAGCCT
401	TTCTGAAAGA	AAATGCCGCC	AAAGACGGCG	TGAAGACCAC	TGCTTCCGGC
451	CTGCAATACA	AAATCACCAA	ACAGGGCGAA	GGCAAACAGC	CGACCAAAGA
501	CGACATCGTT	ACCGTGGAAT	ACGAAGGCCG	CCTGATTGAC	GGTACGGTAT
551	TCGACAGCAG	CAAAGCCAAC	GGCGGCCCGG	TCACCTTCCC	TTTGAGCCAA
601	GTGATTCCGG	GTTGGACCGA	AGGCGTACAG	CTTCTGAAAG	AAGGCGGCGA
651	AGCCACGTTC	TACATCCCGT	CCAACCTTGC	CTACCGCGAA	CAGGGTGCGG
701	GCGACAAAAT	CGGTCCGAAC	GCCACTTTGG	TATTTGATGT	GAAACTGGTC
751	AAAATCGGCG	CACCCGAAAA	CGCGCCCGCC	AAGCAGCCGG	CTCAAGTCGA
801	CATCAAAAAA	GTAAATTAA			

This corresponds to the amino acid sequence <SEQ ID 27; ORF 576-1>:

m576-1.pep

1	MNTIFKISAL	TLSAALALSA	CGKKEAAPAS	ASEPAAASSA	QGDTSSIGST
51	MQQASYAMGV	DIGRSLKQMK	EQGAEIDLKV	FTEAMQAVYD	GKEIKMTEEQ
101	AQEVMMKFLQ	EQQAKAVEKH	KADAKANKEK	GEAFLKENAA	KDGVKTTASG
151	LQYKITKQGE	GKOPTKDDIV	TVEYEGRLID	GTVFDSSKAN	GGPVTFPLSQ
201	VIPGWTEGVQ	LLKEGGEATF	YIPSNLAYRE	QGAGDKIGPN	ATLVFDVKLV
251	KIGAPENAPA	KQPAQVDIKK	VN*		

The following DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 28>:

g576-1.seq

1	ATGAACACCA	TTTTCAAAAT	CAGCGCACTG	ACCCTTTCCG	CCGCTTTGGC
51	ACTTTCCGCC	TGCGGCAAAA	AAGAAGCCGC	CCCCGCATCT	GCATCCGAAC
101	CTGCCGCCGC	TTCTGCCGCG	CAGGGCGACA	CCTTTCAAT	CGGCAGCACG
151	ATGCAGCAGG	CAAGCTATGC	AATGGGCGTG	GACATCGGAC	GCTCCCTGAA
201	ACAAATGAAG	GAACAGGGCG	CGGAAATCGA	TTTGAAAGTC	TTTACCGATG
251	CCATGCAGGC	AGTGTATGAC	GGCAAAGAAA	TCAAATGAC	CGAAGAGCAG
301	GCCCAGGAAG	TGATGATGAA	ATTCTGCAG	GAGCAGCAGG	CTAAAGCCGT
351	AGAAAAACAC	AAGGCGGATG	CGAAGGCCAA	CAAAGAAAAA	GGCGAAGCCT
401	TCCTGAAGGA	AAATGCCGCC	AAAGACGGCG	TGAAGACCAC	TGCTTCCGGT
451	CTGCAGTACA	AAATCACCAA	ACAGGGTGAA	GGCAAACAGC	CGACAAAAGA
501	CGACATCGTT	ACCGTGGAAT	ACGAAGGCCG	CCTGATTGAC	GGTACCGTAT
551	TCGACAGCAG	CAAAGCCAAC	GGCGGCCCGG	CCACCTTCCC	TTTGAGCCAA
601	GTGATTCCGG	GTTGGACCGA	AGGCGTACGG	CTTCTGAAAG	AAGGCGGCGA
651	AGCCACGTTC	TACATCCCGT	CCAACCTTGC	CTACCGCGAA	CAGGGTGCGG
701	GCGAAAAAAT	CGGTCCGAAC	GCCACTTTGG	TATTTGACGT	GAAACTGGTC
751	AAAATCGGCG	CACCCGAAAA	CGCGCCCGCC	AAGCAGCCGG	ATCAAGTCGA

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801 CATCAAAAAA GTAAATTAA

This corresponds to the amino acid sequence <SEQ ID 29; ORF 576-1.ng>:

g576-1.pep

```

1  MNTIFKISAL TLSAALALSA CGKKEAAPAS ASEPAAASAA QGDTSSIGST
51  MQQASYAMGV DIGRSLKQMK EQGAEIDLKV FTDAMQAVYD GKEIKMTEEQ
101 AQEVMMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVKTTASG
151 LQYKITKQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPATFPLSQ
201 VIPGWTEGVR LLKEGGEATF YIPSNLAYRE QGAGEKIGPN ATLVFDVKLV
251 KIGAPENAPA KQPDQVDIKK VN*

```

g576-1/m576-1 ORFs 576-1 and 576-1.ng showed a 97.8% identity in 272 aa overlap

	10	20	30	40	50	60
g576-1.pep	MNTIFKISALTLSAALALSACGKKEAAPASASEPAAASAAQGDTSSIGSTMQQASYAMGV					
m576-1	MNTIFKISALTLSAALALSACGKKEAAPASASEPAAASSAQGDTSSIGSTMQQASYAMGV					
	10	20	30	40	50	60
	70	80	90	100	110	120
g576-1.pep	DIGRSLKQMKEQGAEIDLKVFTDAMQAVYDGKEIKMTEEQAEVMMKFLQEQQAKAVEKH					
m576-1	DIGRSLKQMKEQGAEIDLKVFTDAMQAVYDGKEIKMTEEQAEVMMKFLQEQQAKAVEKH					
	70	80	90	100	110	120
	130	140	150	160	170	180
g576-1.pep	KADAKANKEKGEAFLENAAKDGVKTTASGLQYKITKQEGKQPTKDDIVTVEYEGRLID					
m576-1	KADAKANKEKGEAFLENAAKDGVKTTASGLQYKITKQEGKQPTKDDIVTVEYEGRLID					
	130	140	150	160	170	180
	190	200	210	220	230	240
g576-1.pep	GTVFDSSKANGGPATFPLSQVIPGWTEGVRLLKEGGEATFYIPSNLAYREQGAGEKIGPN					
m576-1	GTVFDSSKANGGPVTFPLSQVIPGWTEGVQLKEGGEATFYIPSNLAYREQGAGDKIGPN					
	190	200	210	220	230	240
	250	260	270			
g576-1.pep	ATLVFDVKLVKIGAPENAPAKQPDQVDIKKVN					
m576-1	ATLVFDVKLVKIGAPENAPAKQPAQVDIKKVN					
	250	260	270			

The following DNA sequence was identified in *N. meningitidis* <SEQ ID 30>:

a576-1.seq

```

1  ATGAACACCA TTTTCAAAAT CAGCGCACTG ACCCTTTCCG CCGCTTTGGC
51  ACTTTCGCC TGCGGCAAAA AAGAAGCCGC CCCCACATCT GCATCCGAAC
101 CTGCCGCCG TTCTTCCGCG CAGGGCGACA CCTCTTCGAT CGGCAGCACG
151 ATGCAGCAGG CAAGCTATGC GATGGGCGTG GACATCGGAC GCTCCCTGAA
201 GCAAATGAAG GAACAGGGCG CGGAAATCGA TTTGAAAGTC TTTACCGAAG
251 CCATGCAGGC AGTGTATGAC GGCAAAGAAA TCAAAATGAC CGAAGAGCAG
301 GCTCAGGAAG TCATGATGAA ATTCCTTCAG GAACAACAGG CTAAGCCGT
351 AGAAAAACAC AAGGCGGACG CGAAGGCCAA TAAAGAAAAA GGCGAAGCCT
401 TTCTGAAAGA AAATGCCGCC AAAGACGGCG TGAAGACCAC TGCTTCCGGC
451 CTGCAATACA AAATCACCAA ACAGGGCGAA GGCAAACAGC CGACCAAAGA
501 CGACATCGTT ACCGTGGAAT ACGAAGGCCG CCTGATTGAC GGTACGGTAT
551 TCGACAGCAG CAAAGCCAAC GGCGGCCCGG TCACCTTCCC TTGAGCCAA
601 GTGATTCTGG GTTGGACCGA AGGCGTACAG CTTCTGAAAG AAGGCGGCGA
651 AGCCACGTTT TACATCCCGT CCAACCTTGC CTACCGCGAA CAGGGTGCGG
701 GCGACAAAAT CGGCCCGAAC GCCACTTTGG TATTGTATGT GAACTGGTG

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751 AAAATCGGCG CACCCGAAAA CGCGCCCGCC AAGCAGCCGG CTAAGTCGA
801 CATCAAAAAA GTAAATTAA

This corresponds to the amino acid sequence <SEQ ID 31; ORF 576-1.a>:

a576-1.pep

1 MNTIFKISAL TLSAALALSA CGKKEAAPAS ASEPAASSA QGDTSSIGST
51 MQQASYAMGV DIGRSLQMK EQGAEIDLKV FTEAMQAVYD GKEIKMTEEQ
101 AQEVMMKFLQ EQQAKAVEKH KADAKANKEK GEAFLENAA KDGVTTFASG
151 LQYKITKQGE GKQPTKDDIV TVEYEGRLID GTVFDSSKAN GGPVTFPLSQ
201 VILGWTEGVQ LLKEGGEATF YIPSNLAYRE QGAGDKIGPN ATLVFDVKLV
251 KIGAPENAPA KQPAQVDIKK VN*

a576-1/m576-1 ORFs 576-1 and 576-1.a 99.6% identity in 272 aa overlap

	10	20	30	40	50	60
a576-1.pep	MNTIFKISALTLSAALALSACGKKEAAPASASEPAAASSAQGDTSSIGSTMQQASYAMGV					
m576-1	MNTIFKISALTLSAALALSACGKKEAAPASASEPAAASSAQGDTSSIGSTMQQASYAMGV					
	10	20	30	40	50	60
	70	80	90	100	110	120
a576-1.pep	DIGRSLQMKEQGAEIDLKVFTTEAMQAVYDGKEIKMTEEQAQEVMMKFLQEQQAKAVEKH					
m576-1	DIGRSLQMKEQGAEIDLKVFTTEAMQAVYDGKEIKMTEEQAQEVMMKFLQEQQAKAVEKH					
	70	80	90	100	110	120
	130	140	150	160	170	180
a576-1.pep	KADAKANKEKGEAFLENAAKDGVTTFASGLQYKITKQGEKQPTKDDIVTVEYEGRLID					
m576-1	KADAKANKEKGEAFLENAAKDGVTTFASGLQYKITKQGEKQPTKDDIVTVEYEGRLID					
	130	140	150	160	170	180
	190	200	210	220	230	240
a576-1.pep	GTVFDSSKANGGPVTFPLSQVILGWTEGVQLLKEGGEATFYIPSNLAYREQGAGDKIGPN					
m576-1	GTVFDSSKANGGPVTFPLSQVILGWTEGVQLLKEGGEATFYIPSNLAYREQGAGDKIGPN					
	190	200	210	220	230	240
	250	260	270			
a576-1.pep	ATLVFDVKLVKIGAPENAPAKQPAQVDIKKVN					
m576-1	ATLVFDVKLVKIGAPENAPAKQPAQVDIKKVN					
	250	260	270			

919 and 919-2

gnm43.seq

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 32>:

m919.seq

1 ATGAAAAAAT ACCTATTCCG CGCCGCCCTG TACGGCATCG CCGCCGCCAT
51 CCTCGCCGCC TGCCAAAGCA AGAGCATCCA AACCTTTCCG CAACCCGACA
101 CATCCGTCAT CAACGGCCCG GACCGGCCGG TCGGCATCCC CGACCCCGCC
151 GGAACGACGG TCGCGGCCGG CGGGGCCGTC TATACCGTTG TACCGCACCT
201 GTCCTGCCC CACTGGGCGG CGCAGGATT TCGCCAAAGC CTGCAATCCT
251 TCCGCCTCGG CTGCGCCAAT TTGAAAAACC GCAAGGCTG GCAGGATGTG
301 TGCGCCCAAG CCTTTCAAAC CCCCCTCCAT TCCTTTCAGG CAAAACAGTT
351 TTTTGAACGC TATTTACGC CGTGGCAGGT TGCAGGCAAC GGAAGCCTTG

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401 CCGGTACGGT TACCGGCTAT TACGAACCGG TGCTGAAGGG CGACGACAGG
451 CGGACGGCAC AAGCCCGCTT CCCGATTAC GGTATTCCCG ACGATTAT
501 CTCGTCCCC CTGCCTGCCG GTTTGCGGAG CGGAAAAGCC CTTGTCCGCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCACAA TCGACAATAC CGGCGGCACA
601 CATAACGCGG ACCTCTCCCG ATTCCCACAT ACCGCGCGCA CAACAGCAAT
651 CAAAGGCAGG TTTGAAGGAA GCCGCTTCCT CCCCTACCAC ACGCGCAACC
701 AAATCAACGG CGGCGCGCTT GACGGCAAAG CCCCGATACT CGGTTACGCC
751 GAAGACCCGT TCGAACTTTT TTTTATGCAC ATCCAAGGCT CGGGCCGTCT
801 GAAAACCCCG TCCGGCAAAT ACATCCGCAT CGGCTATGCC GACAAAAACG
851 AACATCCyTA CGTTTCCATC GGACGCTATA TGGCGGATAA GGGCTACCTC
901 AAATCTGGAC AAACCTCCAT GCAGGGCATT AAGTCTTATA TCGGGCAAAA
951 TCCGCAACGC CTCGCCGAAG TTTTGGGTCA AAACCCAGC TATATCTTTT
1001 TCCGCGAGCT TGCCGGAAGC AGCAATGACG GCCCTGTCGG CGCACTGGGC
1051 ACGCCGCTGA TGGGGGAATA TGCCGCGCA GTCGACCGGC ACTACATTAC
1101 CTTGGGTGCG CCCTTATTG TCGCCACCGC CCATCCGGTT ACCCGCAAAG
1151 CCCTCAACCG CCTGATTATG GCGCAGGATA CCGGCAGCGC GATTAAAGGC
1201 GCGGTGCGCG TGGATTATTT TTGGGGATAC GCGGACGAAG CCGGCGAACT
1251 TGCCGGCAAA CAGAAAACCA CGGATATGT CTGGCAGCTC CTACCCAACG
1301 GTATGAAGCC CGAATACCGC CCGTAA

```

This corresponds to the amino acid sequence <SEQ ID 33; ORF 919>:

m919-pep

```

1 MKKYLFRAL YGIAAAILAA CQSKSIQTFP QPDTSVINGP DRPVGIPDPA
51 GTTVGGGAV YTVVPHLSLP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
101 CAQAFQTPVH SFQAKQFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDDR
151 RTAQARFPIY GIPDDFISVP LPAGLRSGKA LVRIQTGKN SGTIDNTGGT
201 HTADLSRFPI TARTTAIKGR FEGRFLPYH TRNQINGGAL DGKAPILGYA
251 EDPVEFFMH IQSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
301 KLGQTSMQGI KSYMQRNPQR LAEVLGQNPS YIFFRELAGS SNDGPVGALG
351 TPLMGEYAGA VDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*

```

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 34>:

m919-2.seq

```

1 ATGAAAAAAT ACCTATTCCG CGCCGCCCTG TACGGCATCG CCGCCGCCAT
51 CCTCGCGGCC TGCCAAAGCA AGAGCATCCA AACCTTCCG CAACCCGACA
101 CATCCGTCAT CAACGGCCCG GACCGGCCGG TCGGCATCCC CGACCCCGCC
151 GGAACGACGG TCGGCGGCGG CGGGGCCGTC TATACCGTTG TACCGCACCT
201 GTCCCTGCC CACTGGGCGG CGCAGGATT CGCCAAAAGC CTGCAATCCT
251 TCCGCTCGG CTGCGCCAAT TTGAAAAACC GCCAAGGCTG GCAGGATGTG
301 TGCGCCCAAG CCTTTCAAAC CCCCGTCCAT TCCTTTCAGG CAAAACAGTT
351 TTTTGAACGC TATTTACGC CGTGGCAGGT TGCAGGCAAC GGAAGCCTTG
401 CCGGTACGGT TACCGGCTAT TACGAACCGG TGCTGAAGGG CGACGACAGG
451 CGGACGGCAC AAGCCCGCTT CCCGATTAC GGTATTCCCG ACGATTAT
501 CTCGTCCCC CTGCCTGCCG GTTTGCGGAG CGGAAAAGCC CTTGTCCGCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCACAA TCGACAATAC CGGCGGCACA
601 CATAACGCGG ACCTCTCCCG ATTCCCACAT ACCGCGCGCA CAACAGCAAT
651 CAAAGGCAGG TTTGAAGGAA GCCGCTTCCT CCCCTACCAC ACGCGCAACC
701 AAATCAACGG CGGCGCGCTT GACGGCAAAG CCCCGATACT CGGTTACGCC
751 GAAGACCCGT TCGAACTTTT TTTTATGCAC ATCCAAGGCT CGGGCCGTCT
801 GAAAACCCCG TCCGGCAAAT ACATCCGCAT CGGCTATGCC GACAAAAACG
851 AACATCCCTA CGTTTCCATC GGACGCTATA TGGCGGATAA GGGCTACCTC
901 AAATCTGGAC AAACCTCCAT GCAGGGCATT AAGTCTTATA TCGGGCAAAA
951 TCCGCAACGC CTCGCCGAAG TTTTGGGTCA AAACCCAGC TATATCTTTT
1001 TCCGCGAGCT TGCCGGAAGC AGCAATGACG GCCCTGTCGG CGCACTGGGC
1051 ACGCCGCTGA TGGGGGAATA TGCCGCGCA GTCGACCGGC ACTACATTAC
1101 CTTGGGTGCG CCCTTATTG TCGCCACCGC CCATCCGGTT ACCCGCAAAG
1151 CCCTCAACCG CCTGATTATG GCGCAGGATA CCGGCAGCGC GATTAAAGGC

```

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1201 GCGGTGCGCG TGGATTATTT TTGGGGATAC GGCGACGAAG CCGGCCGAAC
 1251 TGCCGGCAAA CAGAAAACCA CGGGATATGT CTGGCAGCTC CTACCCAACG
 1301 GTATGAAGCC CGAATACCGC CCGTAA

This corresponds to the amino acid sequence <SEQ ID 35; ORF 919-2>:

m919-2.pep

1 MKKYLFRAL YGIAAAILAA CQSKIQTFF QPDTSVINGP DRPVGIPDPA
 51 GTTVGGGGAV YTVVPHLSLP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
 101 CAQAFQTPVH SFQAKQFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDDR
 151 RTAQAARFPIY GIPDDFISVP LPAGLRSGKA LVRIRQTGKN SGTIDNTGGT
 201 HTADLSRFPI TARTTAIKGR FEGRFLPYH TRNQINGGAL DGKAPILGYA
 251 EDPVELFFMH IQGSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
 301 KLGQTSMQGI KSYMQRNPQR LAEVLGQNPS YIFFRELAGS SNDGPVAGLG
 351 TPLMGEYAGA VDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
 401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*

The following partial DNA sequence was identified in *N.gonorrhoeae* <SEQ ID 36>:

g919.seq

1 ATGAAAAAAC ACCTGCTCCG CTCCGCCCTG TACGGcatCG CCGCCgccAT
 51 CctcgCCGCC TGCCAAAgca gGAGCATCCA AACCTTTCCG CAACCCGACA
 101 CATCCGTCAT CAACGGCCCG GACCGCCCG CCGGCATCCC CGACCCGCC
 151 GGAACGACGG TTGCCGCGCG CGGGCCGTC TATACCGTTG TGCCGCACCT
 201 GTCCATGCCC CACTGGGCGG CGCaggATTT TGCCAAAGC CTGCAATCCT
 251 TCCGCCTCGG CTGCGCCAAT TTGAAAAACC GCCAAGGCTG GCAGGATGTG
 301 TCGCCCCAAG CCTTTCAAAC CCCCGTGCAT TCCTTTCAGG CAAAGcGgTT
 351 TTTTGAACGC TATTTACGC cgtGGCaggT tgcaggcaAC GGAAGcCTTG
 401 CaggtaaggT TACCGCTAT TACGAACCGG TGCTGAAGGG CGACGGCAGG
 451 CGGACGGAAC GGGCCGCTT CCCGATTAC GGTATTCCCG ACGATTTTAT
 501 CTCGTCCCC CTGCCTGCCG GTTTGCGGGG CGGAAAAAAC CTTGTCCGCA
 551 TCAGGCAGac ggGGA AAAAC AGCGGCACGA TCGACAATGC CGGCGGCACG
 601 CATACCGCCG ACCTCTCCCG ATTCCCATC ACCGCGCGCA CAACGGcaat
 651 caaaGGCAGG TTTGAaggAA GCCGCTTCCT CCCTTACCAC ACGCGCAACC
 701 AAAtcaacGG CGGCgcgcTT GACGGCAAag cccCCATCCT CggttacgcC
 751 GAagaccCcG tcgaacttTT TTTCATGCAC AtccaaggCT CGGGCCGCCT
 801 GAAAACCCcg tccggcaaat acatCCGCaT cggTaagcc gacAAAAACG
 851 AACAtccgTa tgtttccatc ggACGctata TGGCGGACAA AGGCTACCTC
 901 AAGctcgggc agACCTCGAT GCAGGgcatc aaagcCTATA TGCGGCAAAA
 951 TCCGCAACGC CTCGCCGAAG TTTTGGGTCA AAACCCAGC TATATCTTTT
 1001 TCCGCGAGCT TGCCGGAAGC GGCAATGAGG GCCCGTCCG CGCACTGGGC
 1051 ACGCCACTGA TGGGGGAATA CGCCGCGCA ATCGACCGGC ACTACATTAC
 1101 CTTGGGCGCG CCCTTATTG TCGCCACCGC CCATCCGGTT ACCCGCAAAG
 1151 CCCTCAACCG CTTGATTATG GCGCAGGATA CAGGCAGCGC GATCAAAGGC
 1201 CCGGTGCGCG TGGATTATTT TTGGGGTTAC GGCGACGAAG CCGGCCGAAC
 1251 TGCCGGCAAA CAGAAAACCA CGGGATACGT CTGGCAGCTC CTGCCCAACG
 1301 GCATGAAGCC CGAATACCGC CCGTGA

This corresponds to the amino acid sequence <SEQ ID 37; ORF 919.ng>:

g919.pep

1 MKKHLLRSAL YGIAAAILAA CQSRSIQTFF QPDTSVINGP DRPAGIPDPA
 51 GTTVAGGGAV YTVVPHLSMP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
 101 CAQAFQTPVH SFQAKRFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDGR
 151 RTERARFPIY GIPDDFISVP LPAGLRGGKN LVRIRQTGKN SGTIDNAGGT
 201 HTADLSRFPI TARTTAIKGR FEGRFLPYH TRNQINGGAL DGKAPILGYA
 251 EDPVELFFMH IQGSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
 301 KLGQTSMQGI KAYMRNPQR LAEVLGQNPS YIFFRELAGS GNEGVPVAGLG
 351 TPLMGEYAGA IDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
 401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*

a919.seq

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1  ATGAAAAAAT ACCTATCCG CGCCGCCCTG TGCGGCATCG CCGCCGCCAT
51  CCTCGCCGCC TGCCAAAGCA AGAGCATCCA AACCTTTCCG CAACCCGACA
101 CATCCGTCAT CAACGGCCCG GACCGGCCGG TCGGCATCCC CGACCCCGCC
151 GGAACGACGG TCGGGCGCGG CGGGGCCGTT TATACCGTTG TGCCGCACCT
201 GTCCCTGCCC CACTGGGCGG CGCAGGATTT CGCCAAAAGC CTGCAATCCT
251 TCCGCCTCGG CTGCGCCAAT TTGAAAAACC GCCAAGGCTG GCAGGATGTG
301 TCGCCCCAAG CCTTTCAAAC CCCCCTCCAT TCCGTTCAGG CAAAACAGTT
351 TTTTGAACGC TATTTACGCG CGTGGCAGGT TGCAGGCAAC GGAAGCCTTG
401 CCGGTACGGT TACCGGCTAT TACGAGCCGG TGCTGAAGGG CGACGACAGG
451 CGGACGGCAC AAGCCCGCTT CCGGATTTAC GGTATTCCCG ACGATTTTAT
501 CTCCGTCCCC CTGCCTGCCG GTTTGCGGAG CGGAAAAGCC CTTGTCCGCA
551 TCAGGCAGAC GGGAAAAAAC AGCGGCACAA TCGACAATAC CGGCGGCACA
601 CATACGCGCG ACCTCTCCCA ATTCCCCATC ACTGCGCGCA CAACGGCAAT
651 CAAAGGCAGG TTTGAAGGAA GCCGCTTCCT CCCCTACCAC ACGCGCAACC
701 AAATCAACGG CGGCGCGCTT GACGGCAAAG CCCCATACT CGGTTACGCC
751 GAAGACCCCG TCGAATTTT TTTTATGCAC ATCCAAGGCT CGGGCCGTCT
801 GAAAACCCCG TCCGGCAAAT ACATCCGCAT CGGCTATGCC GACAAAACCG
851 AACATCCCTA CGTTTCCATC GGACGCTATA TGGCGGACAA AGGCTACCTC
901 AAGCTCGGGC AGACCTCGAT GCAGGCATC AAAGCCTATA TGCAGCAAAA
951 CCCGCAACGC CTCGCCGAAG TTTTGGGGCA AAACCCAGC TATATCTTTT
1001 TCCGAGAGCT TACCGGAAGC AGCAATGACG GCCTGTCCG CGCACTGGGC
1051 ACGCCGCTGA TGGGCGAGTA CGCCGCGCGA GTCGACCGGC ACTACATTAC
1101 CTTGGGCGCG CCCTTATTTG TCGCCACCGC CCATCCGGTT ACCCGCAAAG
1151 CCCTCAACCG CCGTATTATG GCGCAGGATA CCGCGACGCG GATTAAAGGC
1201 GCGGTGCGCG TGGATTATTT TTGGGGATAC GGCGACGAAG CCGGCGAACT
1251 TGCCGGCAAA CAGAAAACCA CGGGATATGT CTGGCAGCTT CTGCCAACG
1301 GTATGAAGCC CGAATACCGC CCGTAA

```

This corresponds to the amino acid sequence <SEQ ID 39; ORF 919.a>:

a919.pep

```

1  MKKYLFRAL CGIAAAILAA CQSKSIQTFP QPDTSVINGP DRPVGIPDPA
51  GTTVGGGGAV YTVVPHLSLP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
101 CAQAFQTPVH SVQAKQFFER YFTPWQVAGN GSLAGVTGY YEPVLKGDDR
151 RTAQARFPIY GIPDDFISVP LPAGLRSGKA LVRIQTGKN SGTIDNTGGT
201 HTADLSQFPI TARTTAIKGR FEGRSFLPYH TRNQINGGAL DGKAPILGYA
251 EDPVELFFMH IQSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
301 KLGQTSMOGI KAYMQQNPR LAEVLGONPS YIFFRELTGS SNDGPVGALG
351 TPLMGEYAGA VDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*

```

m919/a919 ORFs 919 and 919.a showed a 98.6% identity in 441 aa overlap

```

m919.pep      10      20      30      40      50      60
MKKYLFRALYGI AAILAACQSKSIQTFPQPDTSVINGPDRPVGIPDPAGTTVGGGGAV
|||||
a919          10      20      30      40      50      60
MKKYLFRALCGI AAILAACQSKSIQTFPQPDTSVINGPDRPVGIPDPAGTTVGGGGAV
|||||

m919.pep      70      80      90      100     110     120
YTVVPHLSLPHW AAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHVSFQAKQFFER
|||||
a919          70      80      90      100     110     120
YTVVPHLSLPHW AAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHVSFQAKQFFER
|||||

m919.pep     130     140     150     160     170     180
YFTPWQVAGNGS LAGVTGYEYEPVLKGD RRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
|||||
a919         130     140     150     160     170     180
YFTPWQVAGNGS LAGVTGYEYEPVLKGD RRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
|||||

m919.pep     190     200     210     220     230     240
LVRIQTGKNSGT IDNTGGTHTADLSRFPITARTTAIKGRFEGRSFLPYHTRNQINGGAL
|||||

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a919      LVRIRQTGKNSGTIDNTGGTHTADLSQFPITARTTAIKGRFEGRFLPYHTRNQINGGAL
           190      200      210      220      230      240

           250      260      270      280      290      300
m919.pep  DGKAPILGYAEDPVLEFFMHIIQSGSRLKTPSGKYIRIGYADKNEHPYVSIGRYMADKGYL
           |||||
a919      DGKAPILGYAEDPVLEFFMHIIQSGSRLKTPSGKYIRIGYADKNEHPYVSIGRYMADKGYL
           250      260      270      280      290      300

           310      320      330      340      350      360
m919.pep  KLGQTSMQGIKSYMQRNPQRLAEVLGQNPSYIFFRELAGSSNDGPVGALGTPLMGEYAGA
           |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
a919      KLGQTSMQGIKAYMQNPQRLAEVLGQNPSYIFFRELTGSSNDGPVGALGTPLMGEYAGA
           310      320      330      340      350      360

           370      380      390      400      410      420
m919.pep  VDRHYITLGAPLFVATAHPVTRKALNRLIMAQDTGSAIKGAVRVDFWGYGDEAGELAGK
           |||||
a919      VDRHYITLGAPLFVATAHPVTRKALNRLIMAQDTGSAIKGAVRVDFWGYGDEAGELAGK
           370      380      390      400      410      420

           430      440
m919.pep  QKTTGYVWQLLPNGMKPEYRPX
           |||||
a919      QKTTGYVWQLLPNGMKPEYRPX
           430      440

```

121 and 121-1

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 40>:

m121.seq

```

1  ATGGAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA GCATGGACGG
51  GGCGGATGCC GTACTGATAC GGATGGACGG CGGCAAATGG CTGGGCGCGG
101 AAGGGCACGC CTTTACCCCC TACCCCGCA GGTACGCCG CCAATTGCTG
151 GATTTCAGG ACACAGGCGC AGACGAACTG CACCGCAGCA GGATTTTGTC
201 GCAAGAACTC AGCCGCCTAT ATGCGCAAAC CGCCGCCGAA CTGCTGTGCA
251 GTCAAAACCT CGCACCGTCC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCGAC ACGCGCCGGA ACACGGTTAC AGCATACAGC TTGCCGATTT
351 GCCGCTGCTG GCGxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx
401 xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx
451 xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx
501 xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx
551 xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxx
601 xxxxxCAGC TTCCTTACGA CAAAACGGT GCAAAGTCGG CACAAGGCAA
651 CATATGCCG CAACTGCTCG ACAGGCTGCT CGCCACCCG TATTTGCAC
701 AACGCCACCC TAAAGCACG GGGCGCGAAC TGTTTGCCAT AAATTGGCTC
751 GAAACCTACC TTGACGGCGG CGAAAACCGA TACGACGTAT TGCGGACGCT
801 TTCCCGTTTT ACCGCGCAAA CCGTTTGCGA CGCCGTCTCA CACGACGCGG
851 CAGATGCCCC TCAATGTAC ATTTGCGACG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCCTGCACAG
951 CACCGCCGAC CTGAACCTCG ATCCGCAATG GGTGGAAGCC GCCGnATTG
1001 CGTGGTTGGC GCGGTGTTGG ATTAATCGCA TTCCCGGTAG TCCGCACAAA
1051 GCAACCGGCG CATCCAAACC GTGTATTCTG AnCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 41; ORF 121>:

m121.pep

```

1  METQLYIGIM SGTSMGDADA VLIRMDGGKW LGAEGHAFTP YPGLRRQLL
51  DLQDTGADEL HRSRILSQEL SRLYAQTAAE LLCSQNLAPS DITALGCHGQ

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```

101 TVRHAPHEGY SIQLADLPLL Axxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx
151 xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxxxx
201 xxQLPYDKNG AKSAQGNILP QLLDRLLAHP YFAQRHPKST GRELFAINWL
251 ETYLDGGENR YDVLRTLRF TAQTVCDAYS HAAADARQMY ICDGGIRNPV
301 LMADLAECFG TRVSLHSTAD LNLDPQWVEA AXFAWLAACW INRIPGSPHK
351 ATGASKPCIL XAGYYY*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 42>:

g121.seq

```

1 ATGGAACAC AGCTTTACAT CGGCATTATG TCGGGAACCA GTATGGACGG
51 GGCGGATGCC GTGCTGGTAC GGATGGACGG CGGCAAATGG CTGGGCGCGG
101 AAGGGCACGC CTTTACCCCC TACCCTGACC GGTTGCGCCG CAAATTGCTG
151 GATTTGCAGG ACACAGGCAC AGACGAACTG CACCGCAGCA GGATGTTGTC
201 GCAAGAACTC AGCCGCCTGT ACGCGCAAAC CGCCGCCGAA CTGCTGTGCA
251 GTCAAAACCT CGCTCCGTGC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCCGAC ACGCGCCGGA ACACGGTtac AGCATACAGC TTGCCGATT
351 GCGCTGCTG GCGGAACTGa cgcggatttT TACCGTCggc gacttcCGCA
401 GCCGCGACCT TGCTGCCGCG GGacaAGGTG CGCCGCTCGT CCCCGCCTTT
451 CACGAAGCCC TGTTCCGCGA TGACAGGGAA ACACGCGTGG TACTGAACAT
501 CGGCGGGATT GCCAACATCA GCGTACTCCC CCCCggCGCA CCCGCCTTCG
551 GCTTCGACAC AGGGCCGGGC AATATGCTGA TGGAcgcgtg gacgcaggca
601 cacTGGcagc TGCCTTACGA CAAAacggt gcAAAGgcgg cacAAGGCAA
651 catatTGCcg cAACTGCTCG gcaggctGCT CGCCcaccCG TATTCTCAC
701 AACCCcacc aaAAAGCACG GGgcGCGaac TgtttgcccT AAattggctc
751 gaaacctAcc ttgacggcgg cgaaaaccga tacgacgtat tgcggacgct
801 ttccccgattc accgcgcaaa ccgTttggga cgccgtctca CACGCAGCGG
851 CAGATGCCCG TCAAATGTAC ATTTGCGGCG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCCTGCACAG
951 CACCGCCGAA CTGAACCTCG ATCCTCAATG GGTGGAGGCG gccgCATTtg
1001 cgtggttgC GGCGTGTGG ATTAACCGCA TTCCCGGTAG TCCGCACAAA
1051 GCGACCGCG CATCCAAACC GTGTATTCTG GCGCGGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 43; ORF 121.ng>:

g121.pep

```

1 METQLYIGIM SGTSMDGADA VLVRMDGGKW LGAEGHAFTP YPDRLRRKLL
51 DLQDTGTDEL HRSRMLSQEL SRLYAQTAAE LLCSONLAPC DITALGCHGQ
101 TVRHAPHEGY SIQLADLPLL AELTRIFTVG DFRSRDLAAG GQGAPLVPF
151 HEALFRDDRE TRVVLNIGGI ANISVLPPGA PAFGFDTGPG NMLMDAWTQA
201 HWQLPYDKNG AKAAQGNILP QLLGRLLAHP YFSQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRTLRF TAQTVWDAYS HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAE LNLDPQWVEA AAFAWLAACW INRIPGSPHK
351 ATGASKPCIL GAGYYY*

```

ORF 121 shows 73.5% identity over a 366 aa overlap with a predicted ORF (ORF121.ng) from *N. gonorrhoeae*:

m121/g121

	10	20	30	40	50	60
m121.pep	METQLYIGIMSGTSMDGADAVLIRMDGGKWLGAEGHAFTPYPGRLRRQLLDLQDTGADEL					
g121	METQLYIGIMSGTSMDGADAVLVRMDGGKWLGAEGHAFTPYPDRLRRKLLDLQDTGTDEL					
	10	20	30	40	50	60
	70	80	90	100	110	120
m121.pep	HRSRILSQELSRLYAQTAAELLCSONLAPSDITALGCHGQTVRHAPHEGYSIQLADLPLL					
g121	HRSRMLSQELSRLYAQTAAELLCSONLAPCDITALGCHGQTVRHAPHEGYSIQLADLPLL					
	70	80	90	100	110	120
	130	140	150	160	170	180

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```

m121.pep  AXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
          | : : : : :
g121      AELTRIFTVGDFRSRDLAAGGQGAPLVPAFHEALFRDDRETRVVLNIGGIANISVLPPGA
          130      140      150      160      170      180
          190      200      210      220      230      240
m121.pep  XXXXXXXXXXXXXXXXXXXXXXXQLPYDKNGAKSAQGNILPQLLDRLLAHPYFAQRHPKST
          : : : |||||:||||| |||||:| ||||
g121      PAFGFDTGPGNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLGRLLAHPYFSQPHPKST
          190      200      210      220      230      240
          250      260      270      280      290      300
m121.pep  GRELFAINWLETYLDGGENRYDVLRTLRSRFTAQTVCDVSHAAADARQMYICDGGIRNPV
          |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
g121      GRELFALNWLETYLDGGENRYDVLRTLRSRFTAQTVWDVSHAAADARQMYICGGGIRNPV
          250      260      270      280      290      300
          310      320      330      340      350      360
m121.pep  LMADLAECFGTRVSLHSTADLNLDPOWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL
          |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
g121      LMADLAECFGTRVSLHSTAE LNLDPOWVEAAFAWLAACWINRIPGSPHKATGASKPCIL
          310      320      330      340      350      360

m121.pep  XAGYYYY
          |||||
g121      GAGYYYY

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 44>:

```

a121.seq
1  ATGGAAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA GCATGGACGG
51  GCGGATGCC GTACTGATAC GGATGGACGG CGGCAAATGG CTGGGCGCGG
101 AAGGGCACGC CTTTACCCCC TACCCCGGCA GGTTACGCCG CAAATTGCTG
151 GATTTCAGG ACACAGGCGC GGACGAACTG CACCGCAGCA GGATGTTGTC
201 GCAAGAACTC AGCCGCCTGT ACGCGCAAAC CGCCGCCGAA CTGCTGTGCA
251 GTCAAAACCT CGCGCCGTCC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCAGAC ACGCGCCGGA ACACAGTTAC AGCGTACAGC TTGCCGATTT
351 GCCGCTGCTG GCGGAACGGA CTCAGATTTT TACCGTCGGC GACTTCCGCA
401 GCCCGACCT TGCGGCCGGC GGACAAGGCG CGCCGCTCGT CCCCGCCTTT
451 CACGAAGCCC TGTTCGCGCA CGACAGGGAA ACACGCGCGG TACTGAACAT
501 CGGCGGGATT GCCAACATCA GCGTACTCCC CCCCAGCACA CCCGCCTTCG
551 GCTTCGACAC AGGACCGGGC AATATGCTGA TGGACGCGTG GATGCAGGCA
601 CACTGGCAGC TTCCTTACGA CAAAAACGGT GCAAAGGCGG CACAAGGCAA
651 CATATTGCCG CAACTGCTCG ACAGGCTGCT CGCCACCCG TATTTGCGAC
701 AACCCACCC TAAAAGCACG GGGCGCGAAC TGTTGCCCT AAATTGGCTC
751 GAAACCTACC TTGACGGCGG CGAAAACCGA TACGACGTAT TCGCGACGCT
801 TTCCCGATTC ACCGCGCAA CCGTTTTCGA CGCCGTCTCA CACGCAGCGG
851 CAGATGCCC TCAAATGTAC ATTTGCGGCG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCCTGCACAG
951 CACCGCCGAA CTGAACCTCG ATCCGCAATG GGTAGAAGCC GCCGCGTTCG
1001 CATGGATGGC GCGGTGTTGG GTCAACCGCA TTCCCGGTAG TCCGCACAAA
1051 GCAACCGCG CATCCAAACC GTGTATTCTG GGCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 45; ORF 121.a>:

```

a121.pep
1  METQLYIGIM SGTSMGADA VLIRMDGGKW LGAEGHAFTP YPGRLLRRKLL
51  DLQDTGADEL HRSRMLSQEL SRLYAQTAAE LLCSQNLAPS DITALGCHGQ
101 TVRHAPHSY SVQLADLPLL AERTQIFTVG DFRSRDLAAG GQGAPLVPAF
151 HEALFRDDRE TRAVLNIGGI ANISVLPPDA PAFGFDTPG NMLMDAWMQA
201 HWQLPYDKNG AKAAQGNILP QLLDRLLAHP YFAQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRTLRSR TAQTVFDAVS HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAE LNLDPQWVEA AFAWMAACW VNRIPGSPHK

```


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351 ATGASKPCIL GAGYYY*

m121/a121 ORFs 121 and 121.a 74.0% identity in 366 aa overlap

	10	20	30	40	50	60
m121.pep	METQLYIGIMSGTSMGDADAVLIRMDGGKWLGAEGHAFTYPYGRRLRRQLLDLQDTGADEL					
	: : : : :					
a121	METQLYIGIMSGTSMGDADAVLIRMDGGKWLGAEGHAFTYPYGRRLRRQLLDLQDTGADEL					
	10	20	30	40	50	60
	70	80	90	100	110	120
m121.pep	HRSRILSQELSRLYAQTAELLCSQNLAPSDITALGCHGQTVRHAPENHYSIQLADLPLL					
	: : : : :					
a121	HRSRILSQELSRLYAQTAELLCSQNLAPSDITALGCHGQTVRHAPENHYSIQLADLPLL					
	70	80	90	100	110	120
	130	140	150	160	170	180
m121.pep	AXXX					
	: :					
a121	AERTQIFTVGDFRSRDLAAGGQGAPLVPAPFHEALFRDDRETRAVLNIGGIANISVLPEDA					
	130	140	150	160	170	180
	190	200	210	220	230	240
m121.pep	XXXXXXXXXXXXXXXXXXXXXXXXXQLPYDKNGAKSAQGNILPQLLDRLLAHPYFAQRHPKST					
	: : : : :					
a121	PAFGFDTGPNMLMDAWMQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
	190	200	210	220	230	240
	250	260	270	280	290	300
m121.pep	GRELFALNWLETYLDGGENRYDVLRTLSRFTAQTVCDASHAAADARQMYICDGGIRNPV					
	: : : : :					
a121	GRELFALNWLETYLDGGENRYDVLRTLSRFTAQTVFDAVSHAAADARQMYICGGGIRNPV					
	250	260	270	280	290	300
	310	320	330	340	350	360
m121.pep	LMADLAECFGTRVSLHSTADLNLDQWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL					
	: : : : :					
a121	LMADLAECFGTRVSLHSTAE LNLDQWVEAAAFWMAACWNRIPGSPHKATGASKPCIL					
	310	320	330	340	350	360
m121.pep	XAGYYYY					
a121	GAGYYYY					

Further work revealed the DNA sequence identified in *N. meningitidis* <SEQ ID 46>:

m121-1.seq

1	ATGGAACAC	AGCTTTACAT	CGGCATCATG	TCGGAACCA	GCATGGACGG
51	GGCGGATGCC	GTA CTGATAC	GGATGGACGG	CGGCAAATGG	CTGGGCGCGG
101	AAGGGCACGC	CTTTACCCCC	TACCCCGGCA	GGTTACGCCG	CCAATTGCTG
151	GATTTCAGG	ACACAGGCGC	AGACGAACTG	CACCGCAGCA	GGATTTTGTC
201	GCAAGAACTC	AGCCGCCTAT	ATGCGCAAAC	CGCCGCCGAA	CTGCTGTGCA
251	GTCAAAACCT	CGCACCCTCC	GACATTACCG	CCCTCGGCTG	CCACGGGCAA
301	ACCGTCCGAC	ACGCGCCGGA	ACACGGTTAC	AGCATACAGC	TGCCCGATTT
351	GCCGCTGCTG	GCGGAACGGA	CGCGGATTTT	TACCGTCGGC	GACTTCCGCA
401	GCCGCGACCT	TGCGGCCGGC	GGACAAGGCG	CGCCACTCGT	CCCCGCCTTT
451	CACGAAGCCC	TGTTCCGCGA	CAACAGGGAA	ACACGCGCGG	TACTGAACAT
501	CGGCGGGATT	GCCAACATCA	GCGTACTCCC	CCCCGACGCA	CCCGCCTTCG
551	GCTTCGACAC	AGGGCCGGGC	AATATGCTGA	TGGACGCGTG	GACGCAGGCA
601	CACTGGCAGC	TTCCTTACGA	CAAAAACGGT	GCAAAGGCGG	CACAAGGCAA
651	CATATTGCCG	CAACTGCTCG	ACAGGCTGCT	CGCCCACCCG	TATTTGCGAC
701	AACCCACCC	TAAAAGCACG	GGGCGCGAAC	TGTTTGCCCT	AAATTGGCTC
751	GAAACCTACC	TTGACGGCGG	CGAAAACCGA	TACGACGTAT	TGCGGACGCT

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```

801 TTCCCGTTTT ACCGCGCAAA CCGTTTGCGA CGCCGTCTCA CACGCAGCGG
851 CAGATGCCCG TCAAATGTAC ATTTGCGGCG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGGC ACACGCGTTT CCCTGCACAG
951 CACCGCCGAC CTGAACCTCG ATCCGCAATG GGTGGAAGCC GCCGNATTG
1001 CGTGGTTGGC GCGGTGTTGG ATTAATCGCA TTCCCGGTAG TCCGCACAAA
1051 GCAACCGGCG CATCCAAACC GTGTATTCTG ANCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 47; ORF 121-1>:

```

m121-1.pep
  1 METQLYIGIM SGTSMGDADA VLIRMDGGKW LGAEGHAFTP YPGLRRQLL
 51 DLQDTGADEL HRSRILSQEL SRLYAQTAAE LLCSONLAPS DITALGCHGQ
101 TVRHAPHEGY SIQLADLPLL AERTRIFTVG DFRSRDLAAG GQGAPLVPAF
151 HEALFRDNRE TRAVLNIGGI ANISVLPPDA PAFGFDTPG NMLMDAWTQA
201 HWQLPYDKNG AKAAQGNILP QLLDRLLAHP YFAQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRTLSRF TAQTVCDAYS HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAD LNLDPQWVEA AXFAWLAACW INRIPGSPHK
351 ATGASKPCIL XAGYYY*

```

m121-1/g121 ORFs 121-1 and 121-1.ng showed a 95.6% identity in 366 aa overlap

	10	20	30	40	50	60
m121-1.pep	METQLYIGIMSGTSMGDADAVLIRMDGGKW LGAEGHAFTPYPGLRRQLLDLQDTGADEL					
	: : : : :					
g121	METQLYIGIMSGTSMGDADAVLIRMDGGKW LGAEGHAFTPYPDLRRKLLDLQDTGTDEL					
	10	20	30	40	50	60
	70	80	90	100	110	120
m121-1.pep	HRSRILSQELSRLYAQTAAELLCSONLAPSDITALGCHGQTVRHAPHEGYSIQLADLPLL					
	: : : : :					
g121	HRSRILSQELSRLYAQTAAELLCSONLAPCDITALGCHGQTVRHAPHEGYSIQLADLPLL					
	70	80	90	100	110	120
	130	140	150	160	170	180
m121-1.pep	AERTRIFTVGDFRSRDLAAGGQGAPLVPAFHEALFRDNRETRAVLNIGGIANISVLPPDA					
	: : : : :					
g121	AELTRIFTVGDFRSRDLAAGGQGAPLVPAFHEALFRDDRETRVVLNIGGIANISVLPPGA					
	130	140	150	160	170	180
	190	200	210	220	230	240
m121-1.pep	PAFGFDTPGPNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
	: : : : :					
g121	PAFGFDTPGPNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLGRLLAHPYFSQPHPKST					
	190	200	210	220	230	240
	250	260	270	280	290	300
m121-1.pep	GRELFALNWLETYLDGGENRYDVLRTLSRFTAQTVCDAYSHAAADARQMYICGGGIRNPV					
	: : : : :					
g121	GRELFALNWLETYLDGGENRYDVLRTLSRFTAQTVWDAYSHAAADARQMYICGGGIRNPV					
	250	260	270	280	290	300
	310	320	330	340	350	360
m121-1.pep	LMADLAECFGTRVSLHSTADLNLDPQWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL					
	: : : : :					
g121	LMADLAECFGTRVSLHSTAE LNLDPQWVEAAAFWLAACWINRIPGSPHKATGASKPCIL					
	310	320	330	340	350	360
m121-1.pep	XAGYYYYX					
g121	GAGYYYYX					

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The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 48>:

a121-1.seq

```

1  ATGGAAACAC AGCTTTACAT CGGCATCATG TCGGGAACCA GCATGGACGG
51  GGCGGATGCC GTACTGATAC GGATGGACGG CGGCAAATGG CTGGGCGCGG
101 AAGGGCACGC CTTTACCCCC TACCCCGGCA GGTACGCCG CAAATTGCTG
151 GATTTCAGG ACACAGGCGC GGACGAACTG CACCGCAGCA GGATGTTGTC
201 GCAAGAACTC AGCCGCCTGT ACGCGCAAAC CGCCCGCGAA CTGCTGTGCA
251 GTCAAAACCT CGCGCCGTCC GACATTACCG CCCTCGGCTG CCACGGGCAA
301 ACCGTCAGAC ACGCGCCGGA ACACAGTTAC AGCGTACAGC TTGCCGATTT
351 GCCGCTGCTG GCGGAACGGA CTCAGATTTT TACCGTCGGC GACTTCCGCA
401 GCCGCGACCT TCGGCGCGGC GGACAAGGCG CGCCGCTCGT CCCCGCCTTT
451 CACGAAGCCC TGTTCCGCGA CGACAGGGAA ACACGCGCGG TACTGAACAT
501 CGGCGGGATT GCCAACATCA GCGTACTCCC CCCCGACGCA CCCGCCTTCG
551 GCTTCGACAC AGGACCGGGC AATATGCTGA TGGACGCGTG GATGCAGGCA
601 CACTGGCAGC TTCCTTACGA CAAAACCGT GCAAAGCGG CACAAGGCAA
651 CATATTGCCG CAACTGCTCG ACAGGCTGCT CGCCCACCCG TATTTGCGAC
701 AACCCACCCC TAAAGCACG GGGCGCGAAC TGTTTGCCCT AAATTGGCTC
751 GAAACCTACC TTGACGGCGG CGAAAACCGA TACGACGTAT TGCGGACGCT
801 TTCCCGATTG ACCGCGCAAA CCGTTTTTCA CGCCGTCTCA CACGACGCGG
851 CAGATGCCCC TCAAATGTAC ATTTGCGGCG GCGGCATCCG CAATCCTGTT
901 TTAATGGCGG ATTTGGCAGA ATGTTTCGCG ACACGCGTTT CCCTGCACAG
951 CACCGCCGAA CTGAACCTCG ATCCGCAATG GGTAGAAGCC GCCGCGTTCG
1001 CATGGATGGC GCGGTGTTGG GTCAACCGCA TTCCCGTAG TCCGCACAAA
1051 GCAACCGCG CATCAAACC GTGTATTCTG GCGCGGGAT ATTATTATTG
1101 A

```

This corresponds to the amino acid sequence <SEQ ID 49; ORF 121-1.a>:

a121-1.pep

```

1  METQLYIGIM SGTSMGDADA VLIRMDGGKW LGAEGHAFTF YPGRLLRRLKLL
51  DLQDTGADEL HRSRMLSQEL SRLYAQTAAE LLCSONLAPS DITALGCHGQ
101 TVRHAPESY SVQLADLPLL AERTQIFTVG DFRSRDLAAG GQGAPLVPF
151 HEALFRDDRE TRAVLNIGGI ANISVLPPDA PAFGFDTPG NMLMDAWMQA
201 HWQLPYDKNG AKAAQGNILP QLLDRLLAHP YFAQPHPKST GRELFALNWL
251 ETYLDGGENR YDVLRTLSRF TAQTVFDAVS HAAADARQMY ICGGGIRNPV
301 LMADLAECFG TRVSLHSTAE LNLDPQWVEA AFAFWMAACW VNRIPGSPHK
351 ATGASKPCIL GAGYYY*

```

m121-1/a121-1 ORFs 121-1 and 121-1.a showed a 96.4% identity in 366 aa overlap

	10	20	30	40	50	60
m121-1.pep	METQLYIGIMSGTSMGDADAVLIRMDGGKWLGAEGHAFTFPYPGRLLRRLKLLDLQDTGADEL					
a121-1	METQLYIGIMSGTSMGDADAVLIRMDGGKWLGAEGHAFTFPYPGRLLRRLKLLDLQDTGADEL					
	10	20	30	40	50	60
m121-1.pep	HRSRILSQELSRLYAQTAAELLCSONLAPSDITALGCHGQTVRHAPEHGYSIQLADLPLL					
a121-1	HRSRILSQELSRLYAQTAAELLCSONLAPSDITALGCHGQTVRHAPEHGYSIQLADLPLL					
	70	80	90	100	110	120
m121-1.pep	HRSRILSQELSRLYAQTAAELLCSONLAPSDITALGCHGQTVRHAPEHGYSIQLADLPLL					
a121-1	HRSRILSQELSRLYAQTAAELLCSONLAPSDITALGCHGQTVRHAPEHGYSIQLADLPLL					
	70	80	90	100	110	120
m121-1.pep	AERTRIFTVGDFRSRDLAAGGQGAPLVPFHEALFRDNRETRAVLNIGGIANISVLPPDA					
a121-1	AERTQIFTVGDFRSRDLAAGGQGAPLVPFHEALFRDDRETRAVLNIGGIANISVLPPDA					
	130	140	150	160	170	180
m121-1.pep	AERTRIFTVGDFRSRDLAAGGQGAPLVPFHEALFRDNRETRAVLNIGGIANISVLPPDA					
a121-1	AERTQIFTVGDFRSRDLAAGGQGAPLVPFHEALFRDDRETRAVLNIGGIANISVLPPDA					
	130	140	150	160	170	180
m121-1.pep	PAFGFDTPGPNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
a121-1	PAFGFDTPGPNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
	190	200	210	220	230	240
m121-1.pep	PAFGFDTPGPNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
a121-1	PAFGFDTPGPNMLMDAWTQAHWQLPYDKNGAKAAQGNILPQLLDRLLAHPYFAQPHPKST					
	190	200	210	220	230	240

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	250	260	270	280	290	300
m121-1.pep	GRELFALNWLETYLDGGENRYDVLRTLRSRFTAQTVCDVASHAAADARQMYICGGGIRNPV					
a121-1	GRELFALNWLETYLDGGENRYDVLRTLRSRFTAQTVFVASHAAADARQMYICGGGIRNPV					
	250	260	270	280	290	300
	310	320	330	340	350	360
m121-1.pep	LMADLAECFGTRVSLHSTADLNLDPOWVEAAXFAWLAACWINRIPGSPHKATGASKPCIL					
a121	LMADLAECFGTRVSLHSTAEINLDPOWVEAAAFWMAACWVNRIPGSPHKATGASKPCIL					
	310	320	330	340	350	360
m121-1.pep	XAGYYYYX					
a121	GAGYYYYX					

128 and 128-1

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 50>:

```

m128.seq (partial)
  1  ATGACTGACA ACGCACTGCT CCATTTGGGC GAAGAACCCC GTTTTGATCA
  51  AATCAAAACC GAAGACATCA AACC CGCCCT GCAAACCGCC ATCGCCGAAG
101  CGCGCGAACA AATCGCGGCC ATCAAAGCCC AAACGCACAC CGGCTGGGCA
151  AACACTGTCT AACCCTGAC CGGCATCACC GAACGCGTCG GCAGGATTTG
201  GGGCGTGGTG TCGCACCTCA ACTGCGTCGC CGACACGCCG GAACTGCGCG
251  CCGTCTATAA CGAACTGATG CCCGAAATCA CCGTCTTCTT CACCGAAATC
301  GGACAAGACA TCGAGCTGTA CAACCGCTTC AAAACCATCA AAAATTCCCC
351  CGAATTCGAC ACCCTCTCCC CCGCACAAA AACC AAACTC AACCAC
  1  TACGCCAGCG AAAAACTGCG CGAAGCCAAA TACGCGTTCA GCGAAACCGA
  51  wGTCAAAAAA TAYTTCCCYG TCGGCAAwGT ATTAAACGGA CTGTTTCGCC
101  AAmTCAAAAA ACTmTACGGC ATCGGATTTA CCGAAAAAAC yGTCCCCGTC
151  TGGCACAAG ACGTGCGCTA TtkTGAATTG CAACAAAACG GCGAAmCCAT
201  AGGCGGCGTT TATATGGATT TGTACGCACG CGAAGGCAAA CGCGGCGCGG
251  CGTGGATGAA CGACTACAAA GGCCGCCGCC GTTTTTCAGA CGGCACGCTG
301  CAAyTGCCCA CCGCCTACCT CGTCTGCAAC TTCGCCCCAC CCGTCGGCGG
351  CAGGGAAGCC CGCyTAGCC ACGACGAAAT CCTCATCCTC TTCCACGAAA
401  CCGGACACGG GCTGCACCAC CTGCTTACCC AAGTGGACGA ACTGGGCGTA
451  TCCGGCATCA ACGGCGTAKA ATGGGACGCG GTCGAACTGC CCAGCCAGTT
501  TATGAAAAAT TTCGTTTGGG AATACAATGT CTTGGCACAA mTGTCAGCCC
551  ACGAAGAAAC CGGcgTTCCC yTGCCGAAAG AACTCTTsGA CAAAwTGCTC
601  GCCGCCAAAA ACTTCCAAAG CGGCATGTTC yTsGTCCGGC AAwTGGAGTT
651  CGCCCTCTTT GATATGATGA TTTACAGCGA AGACGACGAA GGCCGTCTGA
701  AAAACTGGCA ACAGGTTTTA GACAGCGTGC GCAAAAAAGT CGCCGTCATC
751  CAGCCGCCCG AATACAACCG CTTTCGCTTG AGCTTCGGCC ACATCTTCGC
801  AGGCGGCTAT TCCGCAGCTn ATTACAGCTA CGCGTGGGCG GAAGTATTGA
851  GCGCGGACGC ATACGCCGCC TTTGAAGAAA GCGACGATGT CGCCGCCACA
901  GGCAAACGCT TTTGGCAGGA AATCCTCGCC GTCGGGGnAT CGCGCAGCGG
951  nGCAGAAATCC TTCAAAGCCT TCCGCGGCCG CGAACCGAGC ATAGACGCAC
1001 TCTTGCGCCA CAGCGGTTTC GACAACGCGG TCTGA

```

This corresponds to the amino acid sequence <SEQ ID 51; ORF 128>:

```

m128.pep (partial)
  1  MTDNALLHLG EEPRFDQIKT EDIKPALQTA IAEAREQIAA IKAQHTTGWA
  51  NTVEPLTGIT ERVGRIWGVV SHLNCVADTP ELRAVYNELM PEITVFFTEI
101  GDIELYNRF KTIKNSPEFD TLSPAQKTKL NH

//
  1  YASEKLREAK YAFSETXVKK YFPVGXVLNG LFAQXKKLYG IGFTEKTVPV

```

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```

51  WHKDVRYXEL QQNGEXIGGV YMDLYAREGK RGGAWMNDYK GRRRFSDGTL
101 QLPTAYLVCN FAPPVGGREA RLSHDEILIL FHETGHGLHH LLTQVDELGV
151 SGINGVXWDA VELPSQFMEN FVWEYNVLAQ XSAHEETGVP LPKELXDKXL
201 AAKNFQXGMF XVRQXEFALF DMMIYSEDDE GRLKNWQQVL DSVRKKVAVI
251 QPPEYNRFAL SFGHIFAGGY SAAXYSYAWA EVLSADAYAA FEESDDVAAT
301 GKRFWQEILA VGXSRSGAES FKAFRGREPS IDALLRHS GF DNAV*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 52>:

g128.seq

```

1  atgattgaca acgCActget ccacttgggc gaagaaccCC GTTTTaatca
51  aatccaaacc gaagACatca AACCCGCCGT CCAAACCGCC ATCGCCGAAG
101 CGCGCGGACA AATCGCCGCC GTCAAAGCGC AAACGCACAC CGGCTGGGCG
151 AACACCGTCG AGCGTCTGAC CGGCATCACC GAACGCGTCG GCAGGATTTG
201 GGGCGTCGTG TCCCATCTCA ACTCCGTCGT CGACACGCCC GAACTGCGCG
251 CCGTCTATAA CGAACTGATG CCTGAAATCA CCGTCTTCTT CACCGAAATC
301 GGACAAGACA TCGAACTGTA CAACCGCTTC AAAACCATCA AAAATTCCCC
351 CGAATTGCA ACGCTTCCCC CGGCACAAAA AACCAAGCTC GATCAGGACC
401 TGCGCGATTT CGTATTGAGC GGCGCGGAAC TGCCGCCCGA ACGGCAGGCA
451 GAACTGGCAA AACTGCAAAC CGAAGGCGCG CAACTTTCCG CCAAATTCTC
501 CCAAAACGTC CTAGACGCGA CGGACGCGTT CGGCATTAC TTTGACGATG
551 CCGCACCCCT TGCCGGCATT CCGAAGACG CGCTCGCCAT GTTTGCCGCC
601 GCCGCGCAAA GCGAAGGCAA AACAGGTTAC AAAATCGGCT TGCAGATTCC
651 GCACTACCTT GCCGTTATCC AATACGCCGG CAACCGCGAA CTGCGCGAAC
701 AAATCTACCG CGCCTACGTT ACCCGTGCCA GCGAACTTTC AAACGACGGC
751 AAATTCGACA ACACCGCCAA CATCGACCGC ACGCTCGAAA ACGCATTGAA
801 AACCGccaaa cTGCTCGGCT TTAAAAATTA CGCCGAATTG TCGCTGGCAA
851 CCAAAATGGC GGACACGCCC GAACAGGTTT TAAACTTCCT GCACGACCTC
901 CCGCGCCGCG CCAAACCTTA CGCCGAAAAA GACCTCGCCG AAGTCAAAGC
951 CTTGCCCCGC GAACACCTCG GTCTCGCCGA CCCGACGCCG TGGGACTTGA
1001 GCTACGCCCG CGAAAACTG CGCGAAGCCA AATACGCATT CAGCGAAACC
1051 GAAGTCAAAA AATACTTCCC CGTCGGCAA GTTCTGGCAG GCCTGTTTCG
1101 CCAAATCAAA AAACCTTACG GCATCGGATT CGCCGAAAAA ACCGTTCCCG
1151 TCTGGCACA AGACGTGCGC TATTTTGAAT TGCAACAAAA CGGCAAAACC
1201 ATCGGCGCGC TTTATATGGA TTTGTACGCA CGCGAAGGCA AACGCGCGCG
1251 CGCGTGGATG AACGActaca AAGGCCGCCG CGGCTTTGCC GACGgcacgc
1301 TGCAACTGCC CACCGCTAC CTCGTCTGCA ACTTCGCCCC GCCCGTCGCG
1351 GGCAAAGAAG CGCGTTTAAG CCACGACGAA ATCCTCACCC TCTTCCACGA
1401 AacCGGCCAC GGACTGCACC ACCTGCTTAC CCAAGTGGAC GAACTGGGCG
1451 TGTCCGGCAT CAacggcgta GAATGGGACG CGGTCGAACT GCCCAGCCAG
1501 TTTATGGAAA ACTTCGTTG GGAATACAAT GTATTGGCAC AAATGTCCCG
1551 CCACGAAGAA AccgGCGAGC CCCTGCCGAA AGAACTCTTC GACAAAATGC
1601 TcgcCGCCAA AAACCTCCAG CGCGGTATGT TCCTCGTCCG GCAAATGGAG
1651 TTCGCCCTCT TCGATATGAT GATTACAGT GAAAGCGACG AATGCCGCTT
1701 GAAAAACTTG CAGCAGGTTT TAGACAGCGT GCGCAAAGAA GTcGCCGTCa
1751 TCCAACCGCC CGAATACAAC CGCTTCGCCA ACAGCTTCGG CCacatctTC
1801 GCcggcGGCT ATTCCGcAGG CTATTACAGC TACGCATGGG CCGAAGTCct
1851 cAGCACCGAT GCCTACGCCG CCTTTGAAGA AAGcGACGac gtcGCCGCCa
1901 CAGGCAAACG CTTCTGGCAA GAAAtccttg ccgtcggegg ctCCCGCAGC
1951 gcgGCGGAAT CCTTCAAAGC CTTCGCGGGA CGCGAACCGA GCATAGACGC
2001 ACTGCTGCGC CAaagcggtT TCGACAACGC gGcttga

```

This corresponds to the amino acid sequence <SEQ ID 53; ORF 128.ng>:

g128.pep

```

1  MIDNALLHLG EEPRFNQIQT EDIKPAVQTA IAEARGQIAA VKAQHTTGWA
51  NTVERLTGIT ERVGRINGVV SHLNSVVDTP ELRAVYNELM PEITVFTEI
101 GQDIELYNRF KTIKNSPEFA TLSPAQKTKL DHDLRDFVLS GAELPPERQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDAAAPLAGI PEDALAMFAA
201 AAQSEGKTGY KIGLQIPHYL AVIQYAGNRE LREQIYRAYV TRASELSNDG

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251 KFDNTANIDR TLENALKTAK LLGFKNYAEL SLATKMADTP EQVLNFLHDL
301 ARRAKPYAEK DLAEVKAFAR EHLGLADPQP WDSL YAGEKL REAKYAFSET
351 EVKKYFPVGK VLAGLFAQIK KLYGIGFAEK TVPVWHKDVR YFELQONGKT
401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFA DGTQLQPTAY LVCNFAPPVG
451 GKEARLSHDE ILTLFHETGH GLHLLTQVD ELGVSGINGV EWDAVELPSQ
501 FMENFVWEYN VLAQMSAHEE TGEPLPKELF DKMLAAKNFQ RGMFLVRQME
551 FALFDMMIYS ESDECRLKNW QQVLDVRKE VAVIQPPEYN RFANSFGHIF
601 AGGYSAGYYS YAWAEVLSTD AYA AFEESDD VAATGKRWFQ EILAVGGSRS
651 AAESFKAFRG REPSIDALLR QSGFDNAA*

```

ORF 128 shows 91.7% identity over a 475 aa overlap with a predicted ORF (ORF 128.ng) from *N. gonorrhoeae*:

m128/g128

	10	20	30	40	50	60
g128.pep	MIDNALLHLGEEPRFNIQTEDIKPAVQTAIAEARGQIAAVKAQTHGTWANTVERLTGIT					
m128	MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIKAQTHGTWANTVEPLTGIT					
	10	20	30	40	50	60
	70	80	90	100	110	120
g128.pep	ERVGRIWGVVSHLNSVVDTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFA					
m128	ERVGRIWGVVSHLNCVADTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD					
	70	80	90	100	110	120
	130	140	150	160	170	180
g128.pep	TLSPAQKTKLDHDLRDFVLSGAELPPERQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
m128	TLSPAQKTKLNH					
	130					
	//					
				340	350	360
g128.pep	YAGEKLREAKYAFSETEVKKYFPVGKVLG					
m128	YASEKLREAKYAFSETXVKKYFPVGXVLNG					
				10	20	30
	370	380	390	400	410	420
g128.pep	LFAQIKKLYGIGFAEKTVPVWHKDVR YFELQONGKTIGGVYMDLYAREGKRGGAWMNDYK					
m128	LFAQXKKLYGIGFTEKTVPVWHKDVR YXELQONGEXIGGVYMDLYAREGKRGGAWMNDYK					
	40	50	60	70	80	90
	430	440	450	460	470	480
g128.pep	GRRRFADGTLQLP TAYLVCNFAPPVGGKEARLSHDEILTLFHETGHGLHLLTQVDELGV					
m128	GRRRFSDGTLQLP TAYLVCNFAPPVGGREARLSHDEILILFHETGHGLHLLTQVDELGV					
	100	110	120	130	140	150
	490	500	510	520	530	540
g128.pep	SGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGEPLPKELFDKMLAAKNFQXGMF					
m128	SGINGVXWDAVELPSQFMENFVWEYNVLAQXSAHEETGVPLPKELXDKMLAAKNFQXGMF					
	160	170	180	190	200	210
	550	560	570	580	590	600

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g128.pep	LVRQMEFALFDMMIYSESEDECR LKNWQVLD SVRKEVAVIQPPEYNRFANSFGHIFAGGY
m128	XVRQXEFALFDMMIYSEDEGR LKNWQVLD SVRKKVAVIQPPEYNRFALSFGHIFAGGY
	220 230 240 250 260 270
g128.pep	610 620 630 640 650 660
	SAGYYSYAWAEVLSTDAYAAFEESDDVAATGKRFWQEILAVGGSRSAAESFKAFRGREPS
m128	SAAXYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGXSRSGAESFKAFRGREPS
	280 290 300 310 320 330
g128.pep	670 679
	IDALLRQSGFDNAAX
m128	IDALLRHSGFDNAVX
	340

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 54>:

a128.seq

1	ATGACTGACA	ACGCACTGCT	CCATTG	GGGC	GAAGAACCCC	GTTTGTATCA
51	AATCAAAACC	GAAGACATCA	AACCCGCCCT	GCAAACCGCC	ATTGCCGAAG	
101	CGCGCGAACA	AATCGCCGCC	ATCAAAGCCC	AAACGCACAC	CGGCTGGGCA	
151	AACACTGTCTG	AACCCCTGAC	CGGCATCACC	GAACGCGTCG	GCAGGATTG	
201	GGGCGTGTTG	TCGCACCTCA	ACTCCGTAC	CGACACGCCC	GAACCTGCGC	
251	CCGCCTACAA	TGAATTAATG	CCGAAATTA	CCGTCTTCTT	CACCGAAATC	
301	GGACAAGACA	TCGAGCTGTA	CAACCGCTTC	AAAACCATCA	AAAACCTCCC	
351	CGAGTTCGAC	ACCCTCTCCC	ACGCGCAAAA	AACCAAACCTC	AACCACGATC	
401	TGCGCGATTT	CGTCCTCAGC	GGCGCGGAAC	TGCCGCCCGA	ACAGCAGGCA	
451	GAATTGGCAA	AACTGCAAAC	CGAAGGCGCG	CAACTTTCGG	CCAAATTCTC	
501	CCAAACGTC	CTAGACGCGA	CCGACGCGTT	CGGCATTAC	TTTGACGATG	
551	CCGACCCGCT	TGCCGGCATT	CCCGAAGACG	CGCTCGCCAT	GTTTGCCGCT	
601	GCCGCGCAAA	GCGAAGGCAA	AACAGGCTAC	AAAATCGGTT	TGCAGATTCC	
651	GCACTACCTC	GCCGTCATCC	AATACGCCGA	CAACCGCAAA	CTGCGCGAAC	
701	AAATCTACCG	CGCTACGTT	ACCCGCGCCA	GCGAGCTTTC	AGACGACGGC	
751	AAATTCGACA	ACACCGCCAA	CATCGACCGC	ACGCTCGAAA	ACGCCCTGCA	
801	AACCGCCAAA	CTGCTCGGCT	TCAAAAATA	CGCCGAATTG	TCGCTGGCAA	
851	CCAAATGGC	GGACACCCCC	GAACAAGTTT	TAAACTTCCT	GCACGACCTC	
901	GCCCGCCGCG	CCAAACCCTA	CGCCGAAAAA	GACCTCGCCG	AAGTCAAAGC	
951	CTTCGCCCGC	GAAAGCCTCG	GCCTCGCCGA	TTTGCAACCG	TGGGACTTGG	
1001	GCTACGCGCG	CGAAAACTG	CGCGAAGCCA	AATACGCATT	CAGCGAAACC	
1051	GAAGTCAAAA	AATACTTCCC	CGTCGGCAAA	GTATTAAACG	GACTGTTCGC	
1101	CCAAATCAAA	AAACTCTACG	GCATCGGATT	TACCGAAAAA	ACCGTCCCCG	
1151	TCTGGCACAA	AGACGTGCGC	TATTTTGAAT	TGCAACAAAA	CGGCGAAACC	
1201	ATAGGCGGCG	TTTATATGGA	TTGTACGCA	CGCGAAGGCA	AACGCGGCGG	
1251	CGCGTGGATG	AACGACTACA	AAGGCCGCCG	CCGTTTTTCA	GACGGCACGC	
1301	TGCAACTGCC	CACCGCCTAC	CTCGTCTGCA	ACTTCACCCC	GCCCCGCGGC	
1351	GGCAAAGAAG	CCCGCTTGAG	CCATGACGAA	ATCCTCACCC	TCTTCCACGA	
1401	AACCGGACAC	GGCCTGCACC	ACCTGCTTAC	CCAAGTCGAC	GAAGTGGGCG	
1451	TATCCGGCAT	CAACGGCGTA	GAATGGGACG	CAGTCGAACT	GCCCAGTCAG	
1501	TTTATGGAAA	ATTTGCTTTG	GGAATACAAT	GTCTTGGCGC	AAATGTCCGC	
1551	CCACGAAGAA	ACCGGCGTTC	CCCTGCCGAA	AGAACTCTTC	GACAAAATGC	
1601	TCGCCGCCAA	AAACTTCCAA	CGCGGAATGT	TCCTCGTCCG	CCAAATGGAG	
1651	TTGCCCTCT	TTGATATGAT	GATTTACAGC	GAAGACGACG	AAGGCCGTCT	
1701	GAAAACTGG	CAACAGGTTT	TAGACAGCGT	GCGCAAAGAA	GTCGCCGTCG	
1751	TCCGACCGCC	CGAATACAAC	CGCTTCGCCA	ACAGCTTCGG	CCACATCTTC	
1801	GCAGGCGGCT	ATTCCGCAGG	CTATTACAGC	TACGCGTGGG	CGGAAGTATT	
1851	GAGCGCGGAC	GCATACGCCG	CCTTTGAAGA	AAGCGACGAT	GTCGCCGCCA	
1901	CAGGCAAACG	CTTTTGGCAG	GAAATCCTCG	CCGTGCGCGG	ATCGCGCAGC	
1951	GCGGCAGAA	CCTTCAAAGC	CTCCGCGGA	CGCGAACCGA	GCATAGACGC	
2001	ACTCTTGCGC	CACAGCGGCT	TCGACAACGC	GGCTTGA		

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This corresponds to the amino acid sequence <SEQ ID 55; ORF 128.a>:

```
a128.pep
1  MTDNALLHLG EEPFRDQIKT EDIKPALQTA IAEAREQIAA IKAQHTGTGWA
51  NTVEPLTGIT ERVGRIWGVV SHLNSVTDTP ELRAAYNELM PEITVFFTEI
101 GQDIELYNRF KTIKNSPEFD TLSHAQKTKL NHDLRDFVLS GAELPPEQQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSEGKTGY KIGLQIPHYL AVIQYADNRK LREQIYRAYV TRASELSDDG
251 KFDNTANIDR TLENALQTAK LLGFKNYAEL SLATKMADTP EQVLNFLHDL
301 ARRAKPYAEK DLAEVKAFAR ESLGLADLQP WDLGYAGEKL REAKYAFSET
351 EVKKYFPVGK VLNGLFAQIK KLYGIGFTEK TVPVWHKDVR YFELQQNGET
401 IGGVYMDLYA REGKRGGAWM NDYKRRRFS DGTQLPTAY LVCNFTPPVG
451 GKEARLSHDE ILTLFHETGH GLHLLTQVD ELGVSGINGV EWDARELPSQ
501 FMENFVWEYN VLAQMSAHEE TGVPLPKELF DKMLAAKNFQ RGMFLVRQME
551 FALFDMMIYS EDDEGRLLKNW QQVLDSVRKE VAVVRPPEYN RFANSFGHIF
601 AGGYSAGYYS YAWAEVLSAD AYAFAESDD VAATGKRWFQ EILAVGGSRS
651 AAESFKAFRG REPSIDALLR HSGFDNAA*
```

m128/a128 ORFs 128 and 128.a showed a 66.0% identity in 677 aa overlap

```
m128.pep      10      20      30      40      50      60
MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAA IKAQHTGTGWTVEPLTGIT
|||||
a128          10      20      30      40      50      60
MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAA IKAQHTGTGWTVEPLTGIT

m128.pep      70      80      90     100     110     120
ERVGRIWGVVSHLNCVADTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD
|||||
a128          70      80      90     100     110     120
ERVGRIWGVVSHLNSVTDTPELRAAYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD

m128.pep      130
TLSPAQKTKLNH-----
|||
a128          130     140     150     160     170     180
TLSHAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY

m128.pep      -----
a128          190     200     210     220     230     240
FDDAAPLAGIPEDALAMFAAAAQSEGKTGYKIGLQIPHYLAVIQYADNRKLREQIYRAYV

m128.pep      -----
a128          250     260     270     280     290     300
TRASELSDDGKFDNTANIDRTLENALQTAKLLGFKNYAELSLATKMADTPEQVLNFLHDL

m128.pep      -----
a128          310     320     330     340     350     360
ARRAKPYAEKDLAEVKAFARESLGLADLQPWDLGYAGEKLREAKYAFSETEVKYFPVGK

m128.pep      160     170     180     190     200     210
VLNGLFAQXKKLYGIGFTEKTVPVWHKDVRYXELQQNGEXIGGVYMDLYAREGKRGGAWM
|||||
a128          370     380     390     400     410     420
VLNGLFAQIKKLYGIGFTEKTVPVWHKDVRYFELQQNGETIGGVYMDLYAREGKRGGAWM

m128.pep      220     230     240     250     260     270
NDYKRRRFS DGTQLPTAYLVCNFAPPVGGREARLSHDEILILFHETGHGLHLLTQVD
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|||||
a128 NDYKGRRRFSDGTLQLPTAYLVCNFTPPVGGKEARLSHDEILTLFHETGHLHLLTQVD
      430      440      450      460      470      480

m128.pep 280      290      300      310      320      330
ELGVSGINGVXWDAVELPSQFMENFVWEYNVLAQXSAHEETGVPLPKELXDKXLAAKNFQ
|||||
a128 ELGVSGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGVPLPKELFDKMLAAKNFQ
      490      500      510      520      530      540

m128.pep 340      350      360      370      380      390
XGMFXVRQXEFALFDMMIYSEDEGRLKNWQQVLDVSRKKVAVIQPPEYNRFALSFGHIF
|||
a128 RGMFLVRQMEFALFDMMIYSEDEGRLKNWQQVLDVSRKEVAVVRPPEYNRFANSFGHIF
      550      560      570      580      590      600

m128.pep 400      410      420      430      440      450
AGGYSAAXYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGXSRSGAESFKAFRG
|||||
a128 AGGYSAGYYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGGSRSAAESFKAFRG
      610      620      630      640      650      660

m128.pep 460      470
REPSIDALLRHSGFDNAVX
|||||
a128 REPSIDALLRHSGFDNAAX
      670

```

Further work revealed the DNA sequence identified in *N. meningitidis* <SEQ ID 56>:

m128-1.seq

```

1 ATGACTGACA ACGCACTGCT CCATTGGGC GAAGAACCCC GTTTTGATCA
51 AATCAAAACC GAAGACATCA AACCCGCCCT GCAAACCGCC ATCGCCGAAG
101 CGCGCGAACA AATCGCGGCC ATCAAAGCCC AAACGCACAC CGGCTGGGCA
151 AACACTGTCTG AACCCTGAC CGGCATCACC GAACGCGTCG GCAGGATTGT
201 GGGCGTGGTG TCGCACCTCA ACTCCGTGCG CGACACGCCC GAACTGCGCG
251 CCGTCTATAA CGAACTGATG CCCGAAATCA CCGTCTTCTT CACCGAAATC
301 GGACAAGACA TCGAGCTGTA CAACCGCTTC AAAACCATCA AAAATTCCCC
351 CGAATTTCGAC ACCCTCTCCC CCGCACAAA AACCAAACTC AACCACGATC
401 TGC GCGATTT CGTCTCAGC GGCGCGGAAC TGCCGCCCGA ACAGCAGGCA
451 GAACTGGCAA AACTGCAAAC CGAAGGCGCG CAACTTTCCG CCAAATTCTC
501 CCAAACGTC CTAGACGCGA CCGACGCGTT CGGCATTAC TTTGACGATG
551 CCGCACCCTG TCGCGGCAAT CCCGAAGACG CGCTCGCCAT GTTTGCCGCC
601 GCCGCGCAA GCGAAAGCAA AACAGGCTAC AAAATCGGCT TGCAGATTCC
651 AACTACCTC GCCGTATCC AATACGCCGA CAACCGCGAA CTGCGCGAAC
701 AAATCTACCG CGCCTACGTT ACCCGCGCCA GCGAACTTTC AGACGACGCG
751 AAATTCGACA ACACCGCCAA CATCGACCGC ACGCTCGCAA ACGCCCTGCA
801 AACCGCCAAA CTGCTCGGCT TCAAAACTA CGCCGAATTG TCGCTGGCAA
851 CCAAAATGGC GGACACGCCC GAACAAGTTT TAAACTTCCT GCACGACCTC
901 GCCCGCCGCG CCAAACCTTA CGCCGAAAAA GACCTCGCCG AAGTCAAAGC
951 CTTGCGCCGC GAAAGCCTGA ACCTCGCCGA TTTGCAACCG TGGGACTTGG
1001 GCTACGCCAG CGAAAACTG CGCGAAGCCA AATACGCGTT CAGCGAAACC
1051 GAAGTCAAAA AACTCTACG GCATCGGATT TACCGAAAAA ACCGTCCCCG
1101 CCAATCAAAA AACTCTACG GCATCGGATT TACCGAAAAA ACCGTCCCCG
1151 TCTGGCACAA AGACGTGCGC TATTTGAAT TGCAACAAA CGCGGAAACC
1201 ATAGGCGGCG TTTATATGGA TTTGTACGCA CGCGAAGGCA AACGCGGCGG
1251 CGCGTGGATG AACGACTACA AAGGCGCGCG CCGTTTTTCA GACGGCACGC
1301 TGCAACTGCC CACCGCTAC CTCGTCTGCA ACTTCGCCCC ACCCGTCCGC
1351 GGCAGGGAAG CCCGCTGAG CCACGACGAA ATCTCATCC TCTTCCACGA
1401 AACCGGACAC GGGCTGCACC ACCTGCTTAC CCAAGTGGAC GAACTGGGCG

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1451 TATCCGGCAT CAACGGCGTA GAATGGGACG CGGTGGAAC T GCCCAGCCAG
1501 TTTATGAAA ATTCGTTTG GGAATACAAT GTCTTGCCAC AAATGTCAGC
1551 CCACGAAGAA ACCGGCGTTC CCTGCCGAA AGAACTCTTC GACAAAATGC
1601 TCGCCGCCAA AACTTCCAA CGCGGCATGT TCCTCGTCCG GCAAATGGAG
1651 TTCGCCCTCT TTGATATGAT GATTACAGC GAAGACGACG AAGGCCGTCT
1701 GAAAACTGG CAACAGGTTT TAGACAGCGT GCGCAAAAAA GTCGCCGTCA
1751 TCCAGCCGCC CGAATACAAC CGCTTCGCCT TGAGCTTCGG CCACATCTTC
1801 GCAGGCGGCT ATTCCGAGG CTATTACAGC TACGCGTGGG CGGAAGTATT
1851 GAGCGCGGAC GCATACGCGC CCTTTGAAGA AAGCGACGAT GTCGCCGCCA
1901 CAGGCAACG CTTTGGCAG GAAATCCTCG CCGTCGGCGG ATCGCGCAGC
1951 GCGGCAGAAT CCTTCAAGC CTTCCGCGGC CGCGAACC GAATAGACGC
2001 ACTCTTGCGC CACAGCGGTT TCGACAACGC GGTCTGA

```

This corresponds to the amino acid sequence <SEQ ID 57; ORF 128-1>:

m128-1.pep.

```

1 MTDNALLHLG EEPFRDQIKT EDIKPALQTA IAEAREQIAA IKAQHTTGWA
51 NTVEPLTGIT ERVGRWGVV SHLNSVADTP ELRAVYNELM PEITVFFTEI
101 GDIELYNRF KTIKNSPEFD TLSPAQTKL NHDLRDFVLS GAELPPEQQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSESKTGY KIGLQIPHYL AVIQYADNRE LREQIYRAYV TRASELSDDG
251 KFDNTANIDR TLNALQTAK LLGFKNYAEL SLATKMADTP EQVLNLFHDL
301 ARRAKPYAEK DLAEVKAFAR ESLNLADLPQ WDLGYASEKL REAKYAFSET
351 EVKKYFPVGK VLNLGFAQIK KLYGIGFTEK TVPVWHKDVR YFELQONGET
401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFS DGTLLQPTAY LVCNFAPPVG
451 GREARLSHDE ILILFHETGH GLHLLTQVD ELGVSGINGV EWDDELPSQ
501 FMENFVWEYN VLAQMSAHEE TGVPLPKELF DKMLAAKNFQ RGMFLVRQME
551 FALFDMMIYS EDDEGRLKNW QQVLDSVRKK VAVIQPPEYN RFALSFGHIF
601 AGGYSAGYYS YAWAEVLSAD AYAAFEESDD VAATGKRFWQ EILAVGGSRS
651 AAESFKAFRG REPSIDALLR HSGFDNAV*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 58>:

g128-1.seq (partial)

```

1 ATGATTGACA ACGCACTGCT CCACTTGGGC GAAGAACCCC GTTTTAATCA
51 AATCAAAACC GAAGACATCA AACCCGCCGT CCAAACCGCC ATCGCCGAAG
101 CGCGCGGACA AATCGCCGCC GTCAAAGCGC AAACGCACAC CGGCTGGGCG
151 AACACCGTCG AGCGTCTGAC CGGCATCACC GAACGCGTCG GCAGGATTGT
201 GGGCGTCGTG TCCCATCTCA ACTCCGTCGT CGACACGCCC GAACTGCGCG
251 CCGTCTATAA CGAACTGATG CCTGAAATCA CCGTCTTCTT CACCGAAATC
301 GGACAAGACA TCGAACTGTA CAACCGCTTC AAAACCATCA AAAATTCCCC
351 CGAATTTGCA ACGCTTTCCC CCGCACAAA AACCAGCTC GATCAGGACC
401 TGCGCGATTT CGTATTGAGC GCGCGGGAAC TGCCGCCCGA ACGGCAGGCA
451 GAACTGGCAA AACTGCAAAC CGAAGGCGCG CAACTTTCCG CCAAATTCTC
501 CCAAAACGTC CTAGACGCGA CCGACGCGTT CGGCATTTAC TTTGACGATG
551 CCGCACCGCT TGCCGGCATT CCCGAAGACG CGCTCGCCAT GTTTGCCGCC
601 GCCGCGCAA GCGAAGGCAA AACAGGTTAC AAAATCGGCT TGCAGATTCC
651 GCACTACCTT GCCGTTATCC AATACGCCGG CAACCGCGAA CTGCGCGAAC
701 AAATCTACCG CGCCTACGTT ACCCGTGCCA GCGAACTTTC AAACGACGGC
751 AAATTCGACA ACACCGCCAA CATCGACCGC ACGCTCGAAA ACGCATTGAA
801 AACCGCCAAA CTGCTCGGCT TAAAAATTA CGCCGAATTG TCGCTGGCAA
851 CCAAATGGC GGACACGCCC GAACAGGTTT TAAACTTCCT GCACGACCTC
901 GCCCGCCGCG CCAAACCTTA CGCCGAAAA GACCTCGCCG AAGTCAAAGC
951 CTTCCGCCGC GAACACCTCG GTCTCGCCA CCCGCAGCCG TGGGACTTGA
1001 GCTACGCCGG CGAAAACTG CGCGAAGCCA AATACGCATT CAGCGAAACC
1051 GAAGTCAAAA AATACTTCCC CGTCGGCAA GTTCTGGCAG GCCTGTTCCG
1101 CCAAATCAA AACTCTACG GCATCGGATT CGCCGAAAA ACCGTTCCCG
1151 TCTGGCACA AGACGTGCGC TATTTTGAAT TGCAACAAA CGGCAAAACC
1201 ATCGCGGCG TTTATATGGA TTTGTACGCA CGCGAAGGCA AACCGGCGG
1251 CGCGTGGATG AACGACTACA AAGGCCGCCG CCGCTTTGCC GACGCGACGC
1301 TGCAACTGCC CACCGCTTAC CTCGTCTGCA ACTTCGCCCC GCCCGTCGGC
1351 GGCAAGAAG CGCGTTTAAG CCACGACGAA ATCCTCACC TCTTCCACGA
1401 AACCGGCCAC GGAAGTACAC ACCTGCTTAC CCAAGTGGAC GAACTGGGCG
1451 TGTCCGCAT CAACGGCGTA AAA

```

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This corresponds to the amino acid sequence <SEQ ID 59; ORF 128-1.ng>:

g128-1.pep (partial)

```

1  MIDNALLHLG EEPFRNQIKT EDIKPAVQTA IAEARGQIAA VKAQHTHTGWA
51  NTVERTLTGIT ERVGRIWGVV SHLNSVVDTP ELRAVYNELM PEITVFFTEI
101 GQDIELYNRF KTIKNSPEFA TLSPAQKTKL DHDLRDFVLS GAELPPERQA
151 ELAKLQTEGA QLSAKFSQNV LDATDAFGIY FDDAAPLAGI PEDALAMFAA
201 AAQSEGKTGY KIGLQIPHYL AVIQYAGNRE LREQIYRAYV TRASELSNDG
251 KFDNTANIDR TLENALKTAK LLGFKNYAEL SLATKMADTP EQVLNFLHDL
301 ARRAKPYAEK DLAEVKAFAR EHLGLADPQP WDLSYAGEKL REAKYAFSET
351 EVKKYFPVGK VLAGLFAQIK KLYGIGFAEK TVPVWHKQVR YFELQQNGKT
401 IGGVYMDLYA REGKRGGAWM NDYKGRRRFA DGTLLQLPTAY LVCNFAPPVG
451 GKEARLSHDE ILTLFHETGH GLHLLTQVD ELGVSGINGV K

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m128-1/g128-1 ORFs 128-1 and 128-1.ng showed a 94.5% identity in 491 aa overlap

	10	20	30	40	50	60
g128-1.pep	MIDNALLHLGEEPRFNQIKTEDIKPAVQTAIAEARGQIAAVKAQHTHTGWANTVERLTGIT					
m128-1	MTDNALLHLGEEPRFDQIKTEDIKPALQTAIAEAREQIAAIKAQHTHTGWANTVEPLTGIT					
	10	20	30	40	50	60
	70	80	90	100	110	120
g128-1.pep	ERVGRIWGVVSHLNSVVDTPPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFA					
m128-1	ERVGRIWGVVSHLNSVADTPPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD					
	70	80	90	100	110	120
	130	140	150	160	170	180
g128-1.pep	TLSPAQKTKLDHDLRDFVLSGAELPPERQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
m128-1	TLSPAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
	130	140	150	160	170	180
	190	200	210	220	230	240
g128-1.pep	FDDAAPLAGIPEDALAMFAAAQSEGKTGYKIGLQIPHYLAVIQYAGNRELREQIYRAYV					
m128-1	FDDAAPLAGIPEDALAMFAAAQSESKTGYKIGLQIPHYLAVIQYADNRELREQIYRAYV					
	190	200	210	220	230	240
	250	260	270	280	290	300
g128-1.pep	TRASELSNDGKFDNTANIDRTLENALKTAKLLGFKNYAELSLATKMADTPEQVLNFLHDL					
m128-1	TRASELSDDGKFDNTANIDRTLANALQTAKLLGFKNYAELSLATKMADTPEQVLNFLHDL					
	250	260	270	280	290	300
	310	320	330	340	350	360
g128-1.pep	ARRAKPYAEKDLAEVKAFAREHLGLADPQPWDLSYAGEKLREAKYAFSETEVKKYFPVGK					
m128-1	ARRAKPYAEKDLAEVKAFARESLNLDLQPDLYASEKLREAKYAFSETEVKKYFPVGK					
	310	320	330	340	350	360
	370	380	390	400	410	420
g128-1.pep	VLAGLFAQIKKLYGIGFAEKTVPVWHKQVRYFELQQNGKTIGGVYMDLYAREGKRGGAWM					
m128-1	VLNGLFAQIKKLYGIGFTEKTVPVWHKQVRYFELQQNGETIGGVYMDLYAREGKRGGAWM					
	370	380	390	400	410	420
	430	440	450	460	470	480
g128-1.pep	NDYKGRRRFADGTLLQLPTAYLVCNFAPPVGGKEARLSHDEILTLFHETGHGLHLLTQVD					

a128-1.seq

a128-1.pep

1	MTDNALLHLG	EEPRFDQIKT	EDIKPALQTA	IAEAREQIAA	IKAQHTGTWA
51	NTVEPLTGIT	ERVGRIWGVV	SHLNSVTDTP	ELRAAYNELM	PEITVFFTEI
101	GQDIELYNRF	KTIKNSPEFD	TLSHAQKTKL	NHDLRDFVLG	GAELPPEQQA
151	ELAKLQTEGA	QLSAKFSQNV	LDATDAFGIY	FDDAAIPLAGI	PEDALAMFQA
201	AAQSEKGTGY	KIGLQIPHYL	AVIQYADNRK	ELAQIYRAYV	TRASELSDDG
251	KFDNTANIDR	TLENALQATAK	LLGFKNYAEL	SLATKMADTP	EQVLNLFHDL

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301  ARRAKPYAEK  DLAEVKAFAR  ESLGLADLQP  WDLGYAGEKL  REAKYAFSET
351  EVKKYFPVGK  VLNGLFAQIK  KLYGIGFTEK  TVPVWHKDVR  YFELQONGET
401  IGGVYMDLYA  REGKRGGAWM  NDYKGRRRFS  DGTLLQLPTAY  LVCNFTPPVG
451  GKEARLSHDE  ILTLFHETGH  GLHLLTQVD  ELGVSGINGV  EWDAVELPSQ
501  FMENFVWEYN  VLAQMSAHEE  TGVPLPKELF  DKMLAAKNFQ  RGMFLVRQME
551  FALFDMMIYS  EDDEGRLKNW  QQVLDSVRKE  VAVVRPPEYN  RFANSFGHIF
601  AGGYSAGYYS  YAWAEVLSAD  AYAAFEESDD  VAATGKRFWQ  EILAVGGSRS
651  AAESFKAFRG  REPSIDALLR  HSGFDNAA*

```

m128-1/a128-1 ORFs 128-1 and 128-1.a showed a 97.8% identity in 677 aa overlap

a128-1.pep	10	20	30	40	50	60
	MTDNALLHLGEEPFRDQIKTEDIKPALQTAIAEAREQIAAIIKAQTHGTWANTVEPLTGIT					
m128-1	MTDNALLHLGEEPFRDQIKTEDIKPALQTAIAEAREQIAAIIKAQTHGTWANTVEPLTGIT					
	10	20	30	40	50	60
a128-1.pep	70	80	90	100	110	120
	ERVGRIGVGVSHLNSVTDTPELRAAYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD					
m128-1	ERVGRIGVGVSHLNSVADTPELRAVYNELMPEITVFFTEIGQDIELYNRFKTIKNSPEFD					
	70	80	90	100	110	120
a128-1.pep	130	140	150	160	170	180
	TLSHAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
m128-1	TLSPAQKTKLNHDLRDFVLSGAELPPEQQAELAKLQTEGAQLSAKFSQNVLDATDAFGIY					
	130	140	150	160	170	180
a128-1.pep	190	200	210	220	230	240
	FDDAAPLAGIPEDALAMFAAAQSEKGTGYKIGLQIPHYLAVIQYADNRKREQIYRAYV					
m128-1	FDDAAPLAGIPEDALAMFAAAQSEKGTGYKIGLQIPHYLAVIQYADNRELREQIYRAYV					
	190	200	210	220	230	240
a128-1.pep	250	260	270	280	290	300
	TRASELSDDGKFDNTANIDRTLENALQTAKLLGFKNYAELSLATKMADTPEQVLNLFHDL					
m128-1	TRASELSDDGKFDNTANIDRTLANALQTAKLLGFKNYAELSLATKMADTPEQVLNLFHDL					
	250	260	270	280	290	300
a128-1.pep	310	320	330	340	350	360
	ARRAKPYAEKDLAEVKAFARESLGLADLQPWDLGYAGEKLREAKYAFSETEVKKYFPVGK					
m128-1	ARRAKPYAEKDLAEVKAFARESLNLADLQPWDLGYASEKLREAKYAFSETEVKKYFPVGK					
	310	320	330	340	350	360
a128-1.pep	370	380	390	400	410	420
	VLNGLFAQIKKLYGIGFTEKTVPVWHKDVRIFYELQONGETIGGVYMDLYAREGKRGGAWM					
m128-1	VLNGLFAQIKKLYGIGFTEKTVPVWHKDVRIFYELQONGETIGGVYMDLYAREGKRGGAWM					
	370	380	390	400	410	420
a128-1.pep	430	440	450	460	470	480
	NDYKGRRRFS DGTLLQLPTAYLVCNFTPPVGGKEARLSHDEILTLFHETGHGLHLLTQVD					
m128-1	NDYKGRRRFS DGTLLQLPTAYLVCNFAPPVGGREARLSHDEILILFHETGHGLHLLTQVD					
	430	440	450	460	470	480
a128-1.pep	490	500	510	520	530	540
	ELGVSGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGVPLPKELFDKMLAAKNFQ					

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m128-1      ELGVSGINGVEWDAVELPSQFMENFVWEYNVLAQMSAHEETGVPLPKELFDKMLAAKNFQ
              490      500      510      520      530      540

              550      560      570      580      590      600
a128-1.pep  RGMFLVRQMEFALFDMMIYSEDDEGR LKNWQQVLD SVRKEVAVVRPPEYNRFANSEFGHIF
              |||||
m128-1      RGMFLVRQMEFALFDMMIYSEDDEGR LKNWQQVLD SVRKKVAVIQPPEYNRFALSFGHIF
              550      560      570      580      590      600

              610      620      630      640      650      660
a128-1.pep  AGGYSAGYYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGGSRSAESFKA FRG
              |||||
m128-1      AGGYSAGYYSYAWAEVLSADAYAAFEESDDVAATGKRFWQEILAVGGSRSAESFKA FRG
              610      620      630      640      650      660

              670      679
a128-1.pep  REPSIDALLRHSGFDNAAX
              |||||
m128-1      REPSIDALLRHSGFDNAVX
              670

```

206

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 62>:

```

m206.seq
1  ATGTTTCCCC CCGACAAAAC CCTTTTCCTC TGTCTCAGCG CACTGCTCCT
51  CGCCTCATGC GGCACGACCT CCGGCAAACA CCGCCAACCG AAACCCAAAC
101 AGACAGTCCG GCAAATCCAA GCCGTCCGCA TCAGCCACAT CGACCGCACA
151 CAAGGCTCGC AGGAACTCAT GCTCCACAGC CTCGGACTCA TCGGCACGCC
201 CTACAAATGG GCGGCGAGCA GCACCGCAAC CGGCTTCGAT TGCAGCGGCA
251 TGATTCAATT CGTTTACAAT AACGCCCTCA ACGTCAAGCT GCCGCGCACC
301 GCCCGCGACA TGGCGGCGGC AAGCCGAAA ATCCCCGACa GCCGCTCAA
351 GGCCGCGGAC CTCGTATTCT TCAACACCGG CGGCGCACAC CGCTACTCAC
401 ACGTCGGACT CTACATCGGC AACGGCGAAT TCATCCATGC CCCCAGCAGC
451 GGCAAAACCA TCAAAACCGA AAAACTCTCC ACACCGTTTT ACGCCAAAAA
501 CTACCTCGGC GCACATACTT TTTTACAGA ATGA

```

This corresponds to the amino acid sequence <SEQ ID 63; ORF 206>:

```

m206.pep.
1  MFPPDKTLFL CLSALLLASC GTTSGKHRQP KPKQTVRQIQ AVRISHIDRT
51  QGSQELMLHS LGLIGTPYKW GGSSTATGFD CSGMIQFVYK NALNVKLPR
101 ARDMAAASRK IPDSRXKAGD LVFFNTGGAH RYSHVGLYIG NGEFIHAPSS
151 GKTIKTEKLS TPFYAKNYLG AHTFFTE*

```

The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 64>:

```

g206.seq
1  atgttttccc ccgacaaaac ccttttcctc tgtctcggcg cactgctcct
51  cgctcatgc ggcacgacct ccggcaaca cgcgaaccg aaacccaaac
101 agacagtccg gcaaatccaa gccgtccgca tcagccacat cggcgcgaca
151 caaggctcgc aggaactcat gctccacagc ctccgactca tcggcacgcc
201 ctacaaatgg ggcggcgagc gcaccgcaac cggcttcgac tgcagcggca
251 tgattcaatt ggtttacaaa aacgccctca acgtcaagct gccgcgcacc
301 gcccgcgaca tggcgcgagg aagccgcaa atccccgaca gccgcctcaa
351 ggccggcgac atcgtattct tcaacacccg cggcgcacac cgctactcac
401 acgtcggact ctacatcggc aacggcgaat tcattccatgc ccccggcagc
451 ggcaaaacca tcaaaaccga aaaactctcc acaccgtttt acgcaaaaaa
501 ctaccttga gcgcatacgt tttttacaga atga

```

ORF 206 shows 96.0% identity over a 177 aa overlap with a predicted ORF (ORF 206.ng) from *N. gonorrhoeae*:

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 66>:

This corresponds to the amino acid sequence <SEQ ID 67; ORF 206.a>:

m206/a206 ORFs 206 and 206.a showed a 99.4% identity in 177 aa overlap

```

              10      20      30      40      50      60
m206.pep  MFPPDKTLFLCLSAALLASCGTTSKGHRQPKPKQTVRQIQAVRISHIDRTQGSQELMLHS
          |||||
a206      MFPPDKTLFLCLSAALLASCGTTSKGHRQPKPKQTVRQIQAVRISHIDRTQGSQELMLHS
              10      20      30      40      50      60

```

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	70	80	90	100	110	120
m206.pep	LGLIGTPYKWGGSSTATGFDCSGMIQFVYKNALNVKLPRRTARDMAAASRKIPDSRXKAGD					
a206	LGLIGTPYKWGGSSTATGFDCSGMIQFVYKNALNVKLPRRTARDMAAASRKIPDSRLKAGD					
	70	80	90	100	110	120
	130	140	150	160	170	
m206.pep	LVFFNTGGAHRYSHVGLYIGNGEFIHAPSSGKTIKTEKLSTPFYAKNYLGAHTFFTEX					
a206	LVFFNTGGAHRYSHVGLYIGNGEFIHAPSSGKTIKTEKLSTPFYAKNYLGAHTFFTEX					
	130	140	150	160	170	

287

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 68>:

m287.seq

1	ATGTTTAAAC	GCAGCGTAAT	CGCAATGGCT	TGTATTTTTG	CCCTTTCAGC
51	CTGCGGGGGC	GGCGGTGGCG	GATCGCCCGA	TGTCAAGTCG	GCGGACACGC
101	TGTCAAAACC	TGCCGCCCT	GTTGTTCTG	AAAAAGAGAC	AGAGGCAAAAG
151	GAAGATGCGC	CACAGGCAGG	TTCTCAAGGA	CAGGGCGCGC	CATCCGCACA
201	AGGCAGTCAA	GATATGGCGG	CGGTTTCGGA	AGAAAATACA	GGCAATGGCG
251	GTGCGGTAA	AGCGGATAAT	CCCAAAATG	AAGACGAGGT	GGCACAAAT
301	GATATGCCGC	AAAATGCCGC	CGGTACAGAT	AGTTCGACAC	CGAATCACAC
351	CCCGGATCCG	AATATGCTTG	CCGGAATAT	GGAAAATCAA	GCAACGGATG
401	CCGGGGAATC	GTCTCAGCCG	GCAAACCAAC	CGGATATGGC	AAATGCGGCG
451	GACGGAATGC	AGGGGGACGA	TCCGTCCGCA	GGCGGGCAAA	ATGCCGGCAA
501	TACGGCTGCC	CAAGGTGCAA	ATCAAGCCGG	AAACAATCAA	GCCGCCGGTT
551	CTTCAGATCC	CATCCCCGCG	TCAAACCCTG	CACCTGCGAA	TGGCGGTAGC
601	AATTTTGAA	GGGTTGATT	GGCTAATGGC	GTTTGTATTG	ACGGGCCGTC
651	GCAAAATATA	ACGTTGACCC	ACTGTAAAGG	CGATTCTTGT	AGTGGCAATA
701	ATTCTTTGA	TGAAGAAGTA	CAGCTAAAAT	CAGAATTTGA	AAAATTAAGT
751	GATGCAGACA	AAATAAGTAA	TTACAAGAAA	GATGGGAAGA	ATGATAAATT
801	TGTCGGTTTG	GTTGCCGATA	GTGTGCAGAT	GAAGGGAATC	AATCAATATA
851	TTATCTTTTA	TAAACCTAAA	CCCACTTCAT	TTGCGCGATT	TAGGCGTTCT
901	GCACGGTCGA	GGCGGTGCGT	TCCGGCCGAG	ATGCCGCTGA	TCCCCGTCAA
951	TCAGGCGGAT	ACGCTGATTG	TCGATGGGGA	AGCGGTCAGC	CTGACGGGGC
1001	ATTCCGGCAA	TATCTTCGCG	CCCGAAGGGA	ATTACCGGTA	TCTGACTTAC
1051	GGGCGGAAA	AATTGCCCGG	CGGATCGTAT	GCCCTTCGTG	TTCAAGGCGA
1101	ACCGGCAAAA	GGCGAAATGC	TTGCGGGCGC	GGCCGTGTAC	AACGGCGAAG
1151	TACTGCATTT	CCATACGGAA	AACGGCCGTC	CGTACCCGAC	CAGGGGCAGG
1201	TTTGCCGCAA	AAGTCGATTT	CGGCAGCAAA	TCTGTGGACG	GCATTATCGA
1251	CAGCGGCGAT	GATTTGCATA	TGGGTACGCA	AAAATTCAAA	GCCGCCATCG
1301	ATGGAAACGG	CTTTAAGGGG	ACTTGGACGG	AAAATGGCAG	CGGGGATGTT
1351	TCCGAAAGT	TTTACGGCCC	GGCCGGCGAG	GAAGTGGCGG	GAAAATACAG
1401	CTATCGCCCC	ACAGATGCGG	AAAAGGGCGG	ATTGCGCGTG	TTTGCCGGCA
1451	AAAAAGAGCA	GGATTGA			

This corresponds to the amino acid sequence <SEQ ID 69; ORF 287>:

m287.pep

1	MFKRSVIAMA	CIFALSACGG	GGGGSPDVKS	ADTLSPKPAAP	VVSEKETEAQ
51	EDAPQAGSQG	QGAPSAQGSQ	DMAAVSEENT	GNGGAVTADN	PKNEDEVAQN
101	DMPQNAAGTD	SSTPNHTPDP	NMLAGNMENQ	ATDAGESSQP	ANQPDMANAA
151	DGMQGDPSA	GGQNAAGTAA	QGANQAGNNQ	AAGSSDPIPA	SNPAPANGGS
201	NFGRVDLANG	VLIDGPSQNI	TLTHCKGDSC	SGNNFLDEEV	QLKSEFEKLS
251	DADKISNYKK	DGKNDKFVGL	VADSVQMKGI	NQYIIFYKPK	PTSFAFRFRS
301	ARSRRSLPAE	MPLIPVQAD	TLIVDGEAVS	LTGHSNIFA	PEGNYRYLTY


```

g287.seq
1 atgtttaaac gcagtgtgat tgcaatggct tgtatttttc ccctttcagc
51 ctgtgggggc ggcggtggcg gatcgcccg tgtcaagtcg gcggacacgc
101 cgtcaaaacc ggccgcccc gttgttgctg aaaaatcgcg ggaaggggtg
151 ctgccgaagc aaaagaaga tgaggagcca gcggcgcggt gcgccgaagc
201 cgatacgtag gacgcaaccg ccggagaagg cagccaagat atgcggcgag
251 ttctggcaga aaatacaggc aatggcggtg cggcaacaac ggacaacccc
301 aaaaatgaag acgcgggggc gcaaaatgat atgcgcgaaa atgcgcgcga
351 atccgcgaat caaacaggga acaaccaacc cgccggttct tcagattccg
401 cccccgcgtc aaacctgcc cctgcgaatg gcggtagcca ttttgaagag
451 acgaacgttg gcaattctgt tgtgattgac ggaccgtcgc aaaaataaac
501 gttgacccac tgtaaaggcg attcttgtaa tggtgataat ttattggatg
551 aagaagcacc gtcaaaatca gaatttgaaa aattaagtga tgaagaaaaa
601 attaaagcag ataaaaaaga cgagcaacgg gagaattttg tcggtttggt
651 tgtcgacagg gtaaaaaagg atggaactaa caaatatata atcttctata
701 cggacaaacc acctactcgt tctgcacggt cagagggttc gcttcggcc
751 gagattccgc tgattcccg tcaatcaggcc gatacgctga ttgtggatgg
801 ggaagcggtc agcctgacgg ggcattccgg caatatcttc gcgccgaag
851 ggaattaccg gtatctgact tacggggcgg aaaaattgcc cgcgggatcg
901 tatgccttcc gtgtgcaggc cgaaccggca aaaggcgaaa tgcttgttgg
951 cacggccgtg tacaacggcg aagtgcctga tttccatatg gaaaacggcc
1001 gtccgtaccc gtccggaggc aggttttgcg caaaagtcga tttcggcagc
1051 aaatctgtgg acggcattat cgacagcggc gatgatttgc atatgggtac
1101 gcaaaaattc aaagccgcga tcgatggaaa cggctttaag gggacttggg
1151 cggaaaaatg cggcggggat gtttcggaa ggttttacgc cccggccggc
1201 gaggaaagtg cgggaaaata cagctatcgc ccgacagatg ctgaaaaggg
1251 cggattccgc gtgtttgccg gcaaaaaaga tcgggattga

```

g287.pep

1	<u>MFKRSVIAMA</u>	<u>CIFPLSACGG</u>	GGGGSPDVKs	ADTPSKPAAP	VVAENAGEGV
51	LPKEKKDEEA	AGGAPQADTQ	DATAGEGSDQ	MAAVSAENTG	NGGAATTDNP
101	KNEDAGAQND	MPQNAAESAN	QTGNNQPAQS	SLSAPASNPA	PANGGSDDEGR
151	TNVGNSVVID	GPSQNTILTH	CKGDSGNGDN	LDDEEAPSKS	EFEKLSDDEK
201	IKRYKKDEQR	ENFVGLVADR	VKKDGTNKYI	IFYTDKPPTR	SARSRRSLPA
251	EPLIPVNQA	DTLIVDGEAV	SLTGHSGNIF	APEGNYRYLT	YGAEKLPGGS
301	YALRVQGEP	A	KGEMLVGTAV	YNGEVLHFHM	ENGRYPYSGG
351	KSLVDGIIDSG	DDLHMGTQKF	KAAIDGNNGF	GTWTENGGGD	VSGRFYGPAG
401	EEVAGKYSYR	PTDAEKGKGGF	VFAGKKDRD*		

```

      10      20      30      40      49
m287.pep  MFKRSVIAMACIFALSACGGGGGGSPDVKSADTL SKPAAPVVSE-----KETEA
          |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
g287      MFKRSVIAMACIFPLSACGGGGGGSPDVKSADTF SKPAAPVVAENAGEGVL PKEKKDEEA
          10      20      30      40      50      60

      50      60      70      80      90      100      109
m287.pep  KEDAPQAGSQGGQGAPSAQGSQDMAAVSEENTGNGGAVTADNPKNEDEVAQN DMPQNAAGT
          |||  :|  |  ::|||  |||||  |||||  |||||  |||||  |||||  |||||
g287      AGGAPQADTQD--ATAGEGSQDMAAVSAENTGNGGAATTDNPKNEDAGAQN DMPQNAA--
          70      80      90      100      110

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m287 . pep	110	120	130	140	150	160	169
	DSSTPNHTPDPNMLAGNMENQATDAGESSQFANQPDMANAADGMQGGDDPSAGGQNAGNTA						
g287	-----						
m287 . pep	170	180	190	200	210	220	229
	AQGANQAGNNQAAGSSDPIPASNPAPANGGSNFRVLDLANGVLIDGPSQNTILTHCKGDS						
	:: : : : : :						
g287	-ESANQTGNQFAGSSDSAPASNPAFANGGSDFGRTNVGNSVVIDGPSQNTILTHCKGDS						
	120	130	140	150	160	170	
m287 . pep	230	240	250	260	270	280	289
	CSGNNFLDEEVQLKSEFEKLSADKISNYKKDGKNDKFVGLVADSVQMKGINQYIIFYKP						
	: : : : : :						
g287	CNGDNLLDEEAPSKSEFEKLSDEEKIKRYKKDEQRENFVGLVADRVKKDGTNKYIIFYTD						
	180	190	200	210	220	230	
m287 . pep	290	300	310	320	330	340	349
	KPTSFAFRRRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLT						
	: :						
g287	KPPT-----RSARSRRSLPAEIPVNPQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLT						
	240	250	260	270	280	290	
m287 . pep	350	360	370	380	390	400	409
	YGAEKLPGGSYALRVQGEPAKGEMLAGAAVYNGEVLHFHTENGRPYPTRGRFAAKVDFGS						
	:						
g287	YGAEKLPGGSYALRVQGEPAKGEMLVGTAVYNGEVLHFHMENGRPYPSGGRFAAKVDFGS						
	300	310	320	330	340	350	
m287 . pep	410	420	430	440	450	460	469
	KSVDGIIDSGDDLHMGTKFKAAIDGNGFKGTWTENGSGDVSGKFYGPAGEEVAGKYSYR						
g287	KSVDGIIDSGDDLHMGTKFKAAIDGNGFKGTWTENGSGDVSGRFYGPAGEEVAGKYSYR						
	360	370	380	390	400	410	
m287 . pep	470	480	489				
	PTDAEKGFGVFAGKKEQDX						
	:						
g287	PTDAEKGFGVFAGKKDRDX						
	420	430					

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 72>:

```

a287 . seq
1  ATGTTTAAAC GCAGTGTGAT TGCAATGGCT TGTATTGTTG CCCTTTTCAGC
51  CTGTGGGGGC GCGCGTGGCG GATCGCCCGA TGTTAAGTCG GCGGACACGC
101 TGTCAAAACC TGCCGCCCTT GTTGTACTG AAGATGTCGG GGAAGAGGTG
151 CTGCCGAAAG AAAAGAAAGA TGAGGAGGCG GTGAGTGGTG CGCCGCAAGC
201 CGATACGCAG GACGCAACCG CCGGAAAAGG CGGTCAAGAT ATGGCGGCAG
251 TTTCGGCAGA AAATACAGGC AATGGCGGTG CGGCAACAAC GGATAATCCC
301 GAAAATAAAG ACGAGGGACC GCAAAATGAT ATGCCGCAAA ATGCCGCCGA
351 TACAGATAGT TCGACACCGA ATCACACCCC TGCACCGAAT ATGCCAACCA
401 GAGATATGGG AAACCAAGCA CCGGATGCCG GGAATCGGC ACAACCGGCA
451 AACCAACCGG ATATGGCAAA TGCGGCGGAC GGAATGCAGG GGGACGATCC
501 GTCGGCAGGG GAAAATGCCG GCAATACGGC AGATCAAGCT GCAAATCAAG
551 CTGAAAACAA TCAAGTCGGC GGCTCTCAA ATCCTGCCTC TTCAACCAAT
601 CCTAACGCCA CGAATGGCGG CAGCGATTTT GGAAGGATAA ATGTAGCTAA
651 TGGCATCAAG CTTGACAGCG GTTCGGAAAA TGTAACGTTG ACACATTGTA
701 AAGACAAAGT ATGCGATAGA GATTTCTTAG ATGAAGAAGC ACCACCAAAA
751 TCAGAATTTG AAAAATTAAG TGATGAAGAA AAAATTAATA AATATAAAAA
801 AGACGAGCAA CGAGAGAATT TTGTCGGTTT GGTGCTGAC AGGGTAGAAA

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a287.ppt

1	MFKRSVIAMA	CIVALSACGG	GGGGSPDVKS	ADTLSKPAAP	VVTEDVGEEV
51	LPKEKKDEEA	VSGAPQADTQ	DATAGKGGQD	MAAVSAENTG	NGGAATTDNP
101	ENKDEGPQND	MPQNAADTDS	STPNHTPAPN	MPTRDMENNQ	PDAGESAQPA
151	NQPDMANAAD	GMQGDPSAG	ENAGNTADQA	ANQAEENNQV	GSQNPASSTN
201	PNATNGGSDF	GRINVANGIK	LDSGSENVTL	THCKDKVCDR	DFLDEEAPPK
251	SEFEKLSDEE	KINKYKKDEQ	RENFVGLVAD	RVEKNGTNKY	VIIYKDKSAS
301	SSSARFRRSA	RSRRSLPAEM	PLIPVNPQADT	LIVDGEAVSL	TGHSGNIFAP
351	EGNYRYLTGY	AEKLSGGSYA	LSVQGEPAKG	EMLAGTAVYN	GEVLHGFHMEN
401	GRPSPSGGRF	AAKVDFGSKS	VDGIIDSGDD	LHMGTQKFKA	VIDNGHFKGT
451	WTENGGGDVS	GRFYGPAGEE	VAGKYSYRPT	DAEKGGEVGF	AGKKKOD*

m287/a287 ORFs 287 and 287.a showed a 77.2% identity in 501 aa overlap

	10	20	30	40	49
m287.pep	MFKRSVIAMACIFALSACGGGGGGSPDVKSADTL SKPAAPV VSE-----				KETEA
a287					
	10	20	30	40	60
	50	60	70	80	100
m287.pep	KEDAPQAGSQQGAPSAQGSQDMAAVSEENTGNGGAVTADNPKNEDEVAQN DMPQNAAGT				109
a287		:		:::	
	70	80	90	100	110
	110	120	130	140	160
m287.pep	DSSTPNHTPDPNMLAGNMENQATDAGESSQPANQPDMANAADGMQGGDDPSAGGQNAGNTA				169
a287			:		
	120	130	140	150	170
	170	180	190	200	220
m287.pep	AQGANQAGNNQAAGSSDPIPASNPAPANGGSNFG RVDLANGVLIDGPSQNITLTHCKGDS				229
a287		:		:	
	180	190	200	210	230
	230	240	250	260	280
m287.pep	CSGNNFLDEEVQLKSEFEKLSADAKISNYKKDKGKDKFVGLVADSVQMKGINQYIIFYKP				289
a287		:		:	
	240	250	260	270	290
	290	300	310	320	340
m287.pep	KP--TSFARFRRSARSRRSLPAEMPLIPVNOADTLIVDGEAVSLTG HSGNIFAPEGNYRY				
		:			

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a287      KSASSSSARFRRSARSRRLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRY
           300      310      320      330      340      350

m287.pep  350      360      370      380      390      400
           LTYGAEKLPGGSYALRVQGEPAKGEMLAGAAVYNGEVLHFHTENGRPYPTRGRFAAKVDF
           ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
a287      LTYGAEKLSGGSYALSVQGEPAKGEMLAGTAVYNGEVLHFHMENGRPSPSGGRFAAKVDF
           360      370      380      390      400      410

m287.pep  410      420      430      440      450      460
           GSKSVLDGIIDSGDDLHMGTKQKFAAIDGNGFKGTWTENGSGDVSQKFGYPAGEEVAGKYS
           ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
a287      GSKSVLDGIIDSGDDLHMGTKQKFAVIDGNGFKGTWTENGSGDVSQKFGYPAGEEVAGKYS
           420      430      440      450      460      470

m287.pep  470      480      489
           YRPTDAEKGFGVFAGKKEQDX
           ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
a287      YRPTDAEKGFGVFAGKKEQDX
           480      490

```

406

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 74>:

m406.seq

```

1  ATGCAAGCAC GGCTGCTGAT ACCTATTCTT TTTTCAGTTT TTATTTTATC
51  CGCCTGCGGG ACACTGACAG GTATTCCATC GCATGGCGGA GGTAACGCT
101 TTGCGGTCGA ACAAGAACTT GTGGCCGCTT CTGCCAGAGC TGCCGTTAA
151 GACATGGATT TACAGGCATT ACACGGACGA AAAGTTGCAT TGTACATTGC
201 CACTATGGGC GACCAAGGTT CAGGCAGTTT GACAGGGGGT CGCTACTCCA
251 TTGATGCACT GATTTCGTGGC GAATACATAA ACAGCCCTGC CGTCCGTACC
301 GATTACACCT ATCCACGTTA CGAAACCACC GCTGAAACAA CATCAGGCGG
351 TTTGACAGGT TTAACCACTT CTTTATCTAC ACTTAATGCC CCTGCACTCT
401 CTCGCACCCA ATCAGACGGT AGCGGAAGTA AAAGCAGTCT GGGCTTAAAT
451 ATTGGCGGGA TGGGGGATTA TCGAAATGAA ACCTTGACGA CTAACCCGCG
501 CGACACTGCC TTCTTTTCCC ACTTGGTACA GACCGTATTT TTCTGCGCG
551 GCATAGACGT TGTTTCTCCT GCCAATGCCG ATACAGATGT GTTTATTAAC
601 ATCGACGTAT TCGGAACGAT ACGCAACAGA ACCGAAATGC ACCTATACAA
651 TGCCGAAACA CTGAAAGCCC AAACAAAAC GGAATATTTC GCAGTAGACA
701 GAACCAATAA AAAATTGCTC ATCAAACCAA AAACCAATGC GTTTGAAGCT
751 GCCTATAAAG AAAATTACGC ATTGTGGATG GGGCCGTATA AAGTAAGCAA
801 AGGAATTAAG CCGACGGAAG GATTAATGGT CGATTCTCC GATATCCGAC
851 CATACGGCAA TCATACGGGT AACTCCGCC CATCCGTAGA GGCTGATAAC
901 AGTCATGAGG GGTATGGATA CAGCGATGAA GTAGTGCAC AACATAGACA
951 AGGACAACCT TGA

```

This corresponds to the amino acid sequence <SEQ ID 75; ORF 406>:

m406.pep

```

1  MQARLLIPIL FSVFILSACG TLTGIPSHGG GKRFAVEQEL VAASARAAVK
51  DMDLQALHGR KVALYIATMG DQSGSLTGG RYSIDALIRG EYINSPAVRT
101 DYTYPRYETT AETTSGLTG LTSLSTLNA PALSRQSDG SGSKSSLGLN
151 IGMGDYRNE TLTNPRDTA FLSHLVQTVF FLRGIDVVSP ANADTDVFIN
201 IDVFGTIRNR TEMHLYNAET LKAQTKLEYF AVDRTNKKLL IKPKTNAFEA
251 AYKENYALWM GPYKVSKEIK PTEGLMVDFS DIRPYGNHTG NSAPSVEADN
301 SHEGYGYSDE VVRQHRQGQP *

```

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The following partial DNA sequence was identified in *N. gonorrhoeae* <SEQ ID 76>:

g406.seq

```

1  ATGCGGGCAC GGCTGCTGAT ACCTATTCTT TTTTCAGTTT TTATTTTATC
51  CGCCTGCGGG AACTGACAG GTATTCCATC GCATGGCGGA GGCAAACGCT
101 TCGCGGTGCA ACAAGAACTT GTGGCCGCTT CTGCCAGAGC TGCCGTTAAA
151 GACATGGATT TACAGGCATT ACACGGACGA AAAGTTGCAT TGTACATTGC
201 AACTATGGGC GACCAAGGTT CAGGCAGTTT GACAGGGGGT CGCTACTCCA
251 TTGATGCACT GATTGCGGGC GAATACATAA ACAGCCCTGC CGTCCGCACC
301 GATTACACCT ATCCGCGTTA CGAAACCACC GCTGAAACAA CATCAGGCGG
351 TTTGACGGGT TTAACCACTT CTTTATCTAC ACTTAATGCC CCTGCACTCT
401 CGCGCACCCA ATCAGACGGT AGCGGAAGTA GGAGCAGTCT GGGCTTAAAT
451 ATTGGCGGGA TGGGGGATTA TCGAAATGAA ACCTTGACGA CCAACCCGCG
501 CGACACTGCC TTTCTTTCCC ACTTGGTGCA GACCGTATTT TTCCTGCGCG
551 GCATAGACGT TGTTTCTCCT GCCAATGCCG ATACAGATGT GTTTATTAAC
601 ATCGACGTAT TCGGAACGAT ACGCAACAGA ACCGAAATGC ACCTATACAA
651 TGCCGAAACA CTGAAAGCCC AAACAAACT GGAATATTTC GCAGTAGACA
701 GAACCAATAA AAAATTGCTC ATCAAACCCA AAACCAATGC GTTTGAAGCT
751 GCCTATAAAG AAAATTACGC ATTGTGGATG GGGCCGTATA AAGTAAGCAA
801 AGGAATCAAA CCGACGGAAG GATTGATGGT CGATTTCTCC GATATCCAAC
851 CATACGGCAA TCATACGGGT AACTCCGCCC CATCCGTAGA GGCTGATAAC
901 AGTCATGAGG GGTATGGATA CAGCGATGAA GCAGTGCAGC AACATAGACA
951 AGGGCAACCT TGA

```

This corresponds to the amino acid sequence <SEQ ID 77; ORF 406.ng>:

g406.pep

```

1  MRARLLIPIL FSVFILSACG TLTGIPSHGG GKRFVEQEL VAASARAANK
51  DMDLQALHGR KVALYIATMG DQSGSLTGG RYSIDALIRG EYINSPAVRT
101 DYTYPREYET AETTSGLTGT LTSLSTLNA PALSRQSDG SGSSRLGLN
151 IGGMGDYRNE TLTNNPRDTA FLSHLVQTVF FLRGIDVVSF ANADTVFIN
201 IDVFGTIRNR TEMHLYNAET LKAQTKLEYF AVDRNTNKKL IKPKTNAFEK
251 AYKENYALWM GPYKVSKEIK PTEGLMVDFS DIQPYGNHTG NSAPSVEADN
301 SHEGYGYSDE AVRQHRQGP *

```

ORF 406.ng shows 98.8% identity over a 320 aa overlap with a predicted ORF (ORF406.a) from *N. gonorrhoeae*:

g406/m406

	10	20	30	40	50	60
g406.pep	MRARLLIPILFSVFILSACGLTGIPSHGGGKRFVEQELVAASARAANKDMDLQALHGR					
m406	MQARLLIPILFSVFILSACGLTGIPSHGGGKRFVEQELVAASARAANKDMDLQALHGR					
	10	20	30	40	50	60
	70	80	90	100	110	120
g406.pep	KVALYIATMGDQSGSLTGGRYSIDALIRGEYINSPAVRTDYTYPREYETAEETSGGLTG					
m406	KVALYIATMGDQSGSLTGGRYSIDALIRGEYINSPAVRTDYTYPREYETAEETSGGLTG					
	70	80	90	100	110	120
	130	140	150	160	170	180
g406.pep	LTSLSTLNAPALSRQSDGSGSSRLGLNIGMGDYRNETLTNNPRDTAFLSHLVQTVF					
m406	LTSLSTLNAPALSRQSDGSGSKSSLGLNIGMGDYRNETLTNNPRDTAFLSHLVQTVF					
	130	140	150	160	170	180
	190	200	210	220	230	240

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```

g406.pep  FLRGIDVVSPANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
|||||
m406      FLRGIDVVSPANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
              190      200      210      220      230      240

              250      260      270      280      290      300
g406.pep  IKPKTNAFEAAAYKENYALWMGPYKVS KGIKPTEGLMVDFS DIQPYGNHTGNSAPSVEADN
|||||
m406      IKPKTNAFEAAAYKENYALWMGPYKVS KGIKPTEGLMVDFS DIRPYGNHTGNSAPSVEADN
              250      260      270      280      290      300

              310      320
g406.pep  SHEGYGYSDEAVRQHRQGPX
|||||
m406      SHEGYGYSDEVVRQHRQGPX
              310      320

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 78>:

```

a406.seq
1  ATGCAAGCAC GGCTGCTGAT ACCTATTCTT TTTTCAGTTT TTATTTTATC
51  CGCCTGCGGG ACAC TGACAG GTATTCCATC GCATGGCGGA GGTAAACGCT
101 TCGCGGTCGA ACAAGAACTT GTGGCCGCTT CTGCCAGAGC TGCCGTTAAA
151 GACATGGATT TACAGGCATT ACACGGACGA AAAGTTGCAT TGTACATTGC
201 AACTATGGGC GACCAAGGTT CAGGCAGTTT GACAGGGGGT CGCTACTCCA
251 TTGATGCACT GATTCTGTCG GAATACATAA ACAGCCCTGC CGTCCGTACC
301 GATTACACCT ATCCACGTTA CGAAACCACC GCTGAAACAA CATCAGGCGG
351 TTTGACAGGT TTAACCACTT CTTTATCTAC ACTTAATGCC CCTGCACTCT
401 CGCGCACCCA ATCAGACGGT AGCGGAAGTA AAAGCAGTCT GGGCTTAAAT
451 ATTGGCGGGA TGGGGGATTA TCGAAATGAA ACCTTGACGA CTAACCCCGC
501 CGACACTGCC TTTCTTTCCC ACTTGGTACA GACCGTATTT TTCCTGCGCG
551 GCATAGACGT TGTTTCTCCT GCCAATGCCG ATACGGATGT GTTTATTAAAC
601 ATCGACGTAT TCGGAACGAT ACGCAACAGA ACCGAAATGC ACCTATACAA
651 TGCCGAAACA CTGAAAGCCC AAACAAAAC TGAATATTTT GCAGTAGACA
701 GAACCAATAA AAAATTGCTC ATCAAACCAA AAACCAATGC GTTTGAAGCT
751 GCCTATAAAG AAAATTACGC ATTGTGGATG GGACCGTATA AAGTAAGCAA
801 AGGAATTAAA CCGACAGAAG GATTAATGGT CGATTTCTCC GATATCCAAC
851 CATACGGCAA TCATATGGGT AACTCTGCCC CATCCGTAGA GGCTGATAAC
901 AGTCATGAGG GGTATGGATA CAGCGATGAA GCAGTGCAC GACATAGACA
951 AGGGCAACCT TGA

```

This corresponds to the amino acid sequence <SEQ ID 79; ORF 406.a>:

```

a406.pep
1  MQARLLIPIL FSVFILSACG TLTGIPSHGG GKRF AVEQEL VAASARA AVK
51  DMDLQALHGR KVALYIATMG DQSGSLTGG RYSIDALIRG EYINSPAVRT
101 DYTPRYETT AETTSGLTG LTSLSTLNA PALSR TQSDG SGSSSLGLN
151 IGMGDYRNE TLTTNPRDTA FLSHLVQTVF FLRGIDVVSP ANADTDVFIN
201 IDVFGTIRNR TEMHLYNAET LKAQTKLEYF AVDR TNKKLL IKPKTNAFEA
251 AYKENYALWM GPYKVS KGIK PTEGLMVDFS DIQPYGNHMG NSAPSVEADN
301 SHEGYGYSDE AVRRHRQGP *

m406/a406  ORFs 406 and 406.a showed a 98.8% identity in 320 aa overlap

              10      20      30      40      50      60
m406.pep  MQARLLIPILFSVFILSACGTLTGIPSHGGGKRF AVEQELVAASARA AVK DMDLQALHGR
|||||
a406      MQARLLIPILFSVFILSACGTLTGIPSHGGGKRF AVEQELVAASARA AVK DMDLQALHGR
              10      20      30      40      50      60

              70      80      90      100     110     120
m406.pep  KVALYIATMGDQSGSLTGGRYSIDALIRGEYINSPAVRTDYTPRYETTAETTSGLTG
|||||

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a406	KVALYIATMGDQSGSLTGGRYSIDALIRGEYINSPAVRTDYTPRYETTAETTSGGLTG
	70 80 90 100 110 120
m406.pep	130 140 150 160 170 180
	LTTSLSLTLNAPALSRTQSDGSGSKSSLGLNIGGMGDYRNETLTNPRDTAFLSHLVQTVF
a406	LTTSLSLTLNAPALSRTQSDGSGSKSSLGLNIGGMGDYRNETLTNPRDTAFLSHLVQTVF
	130 140 150 160 170 180
m406.pep	190 200 210 220 230 240
	FLRGIDVVSPANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
a406	FLRGIDVVSPANADTDVFINIDVFGTIRNRTEMHLYNAETLKAQTKLEYFAVDRTNKKLL
	190 200 210 220 230 240
m406.pep	250 260 270 280 290 300
	IKPKTNAFEAAAYKENYALWMGPYKVS KG I KPTEGLMVD FSDIRPYGNHTGNSAPSVEADN
a406	IKPKTNAFEAAAYKENYALWMGPYKVS KG I KPTEGLMVD FSDIQPYGNHMGNSAPSVEADN
	250 260 270 280 290 300
m406.pep	310 320
	SHEGYGYSDEVVRQHRQGQPK
a406	SHEGYGYSDEAVRRHRQGQPK
	310 320

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 80>:

m726.seq

1	ATGACCATCT	ATTCAAAAA	CGGCTTTTAC	GACGACACAT	TGGGCGGCAT
51	CCCCGAAGGC	GCGGTTGCCG	TCCGCGCCGA	AGAATACGCC	GCCCTTTTGG
101	CAGGACAGGC	GCAGGGCGGG	CAGATTGCCG	CAGATTCCGA	CGGCCGCCCC
151	GTTTTAACCC	CGCCGCGCCC	GTCCGATTAC	CACGAATGGG	ACGGCAAAAA
201	ATGGAAAATC	AGCAAAGCCG	CCGCCGCCGC	CCGTTTCGCC	AAACAAAAAA
251	CCGCCTTGGC	ATTCCGCCTC	GCGGAAAAGG	CGGACGAACT	CAAAAACAGC
301	CTCTTGCGCG	GCTATCCCCA	AGTGGAATC	GACAGCTTTT	ACAGGCAGGA
351	AAAAGAAGCC	CTCGCGCGGC	AGGCGGACAA	CAACGCCCCG	ACCCCGATGC
401	TGGCGCAAAT	CGCCGCCGCA	AGGGGCGTGG	AATTGGACGT	TTTGATTGAA
451	AAAGTTATCG	AAAAATCCGC	CCGCCTGGCT	GTTGCCGCGG	GCGCGATTAT
501	CGGAAAGCGT	CAGCAGCTCG	AAGACAAATT	GAACACCATC	GAAACCGCGC
551	CCGGATTGGA	CGCGCTGGAA	AAGGAAATCG	AAGAATGGAC	GCTAAACATC
601	GGCTGA				

This corresponds to the amino acid sequence <SEQ ID 81; ORF 726>:

m726.pep

1	MTIYFKNGFY	DDTLGGIPEG	AVAVRAEEYA	ALLAGQAQGG	QIAADSDGRP
51	VLTPPRPSDY	HEWDGKKWKI	SKAAAAARFA	KQKTALAFRL	AEKADELKNS
101	LLAGYPQVEI	DSFYRQEKEA	LARQADNNAP	TPMLAQIAAA	RGVELDVLIE
151	KVIEKSARLA	VAAGAIIGKR	QQLDKLNTI	ETAPGLDALE	KEIEEWTLNI
201	G*				

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 82>:

m907-2.seq

1	ATGAGAAAAC	CGACCGATAC	CCTACCCGTT	AATCTGCAAC	GCCGCCGCGT
51	GTTGTGTGCC	GCCGGTGCGT	TGTTGCTCAG	TCCTCTGGCG	CACGCCGCGG
101	CGCAACGTGA	GGAAACGCTT	GCCGACGATG	TGGCTTCCGT	GATGAGGAGT

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151 TCTGTCGGCA GCGTCAATCC GCCGAGGCTG GTGTTTGACA ATCCGAAAGA
201 GGGCGAGCGT TGGTTGTCTG CCATGTCGGC ACGTTTGCCA AGSTTCGTCC
251 CCGAGGAGGA GGAGCGGCGC AGGCTGCTGG TCAATATCCA GTACGAAAGC
301 AGCCGGGCGG GTTTGGATAC GCAGATTGTG TTGGGGCTGA TTGAGGTGGA
351 AAGCGCGTTC CGCCAGTATG CAATCAGCGG TGTCGGCGCG CGCGGCTGA
401 TGCAGGTAT GCCGTTTGG AAAAATAACA TCGGCAAACC GGCGACAAC
451 CTGTTGACA TCCGCACCAA CCTGCGTTAC GGCTGTACCA TCCTGCGCCA
501 TTACCGGAAT CTTGAAAAAG GCAACATCGT CCGCGCGCTT GCCCGCTTTA
551 ACGGCAGCTT GGGCAGCAAT AAATATCCGA ACGCCGTTT GGGCGCGTGG
601 CGCAACCGCT GGCAGTGGCG TTGA

```

This corresponds to the amino acid sequence <SEQ ID 83; ORF 907-2>:

m907-2.pep

```

1 MRKPTDTLPV NLQRRRLCA AGALLLSPLA HAGAOREETL ADDVASVMRS
51 SVGSVNPRL VFDNPKGER WLSAMSARLA RFVPEEEERR RLLVNIQYES
101 SRAGLDTQIV LGLIEVESAF RQYAISGVGA RGLMQVMPFW KNYIGKPAHN
151 LFDIRTNLRY GCTILRHYN LEKGNIVRAL ARFNGSLGSN KYPNAVLGAW
201 RNRWQWR*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 84>:

m953.seq

```

1 ATGAAAAAA TCATCTTCGC CGCACTCGCA GCCGCCGCCA TCAGTACTGC
51 CTCCGCCGCC ACCTACAAAG TGGACGAATA TCACGCCAAC GCCCGTTTCG
101 CCATCGACCA TTTCAACACC AGCACCAACG TCGGCGGTTT TTACGGTCTG
151 ACCGGTTCGG TCGAGTTCGA CCAAGCAAAA CGCGACGTA AAATCGACAT
201 CACCATCCC ATTGCCAACC TGCAAAGCGG TTCGCAACAC TTTACCGACC
251 ACCTGAAATC AGCCGACATC TTCGATGCCG CCCAATATCC GGACATCCGC
301 TTTGTTTCCA CCAAATTCAT CTTCAACGGC AAAAATACTG TTTCCGTTGA
351 CGGCAACCTG ACCATGCACG GCAAAACGCG CCCCGTCAA CTCAAAGCCG
401 AAAAATTCAT CTGCTACCAA AGCCCGATGG AGAAAACCGA AGTTTGTGGC
451 GGCGACTTCA GCACCACCAT CGACCGCACC AAATGGGGCA TGGACTACCT
501 CGTTAACGTT GGTATGACCA AAAGCGTCCG CATCGACATC CAAATCGAGG
551 CAGCCAAACA ATAA

```

This corresponds to the amino acid sequence <SEQ ID 85; ORF 953>:

m953.pep

```

1 MKKIIFAALA AAAISTASAA TYKVDEYHAN ARFAIDHFNT STNVGGFYGL
51 TGSVEFDQAK RDGKIDITIP IANLQSGSQH FTDHLKSADI FDAQYPPDIR
101 FVSTKFNENG KKLVSVDGNL TMHGKTAPVK LKAKEFNCYQ SPMEKTEVCG
151 GDFSTTIDRT KWGMDYLVNV GMTKSVRIDI QIEAAKQ*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 86>:

orf1-1.seq

```

1 ATGAAAACAA CCGACAAACG GACAACCGAA ACACACCGCA AAGCCCCGAA
51 AACCGGCCGC ATCCGCTTCT CGCCTGCTTA CTTAGCCATA TGCTGTCTGT
101 TCGGCATTCT TCCCCAAGCC TGGGCGGGAC ACATTATTT CGGCATCAAC
151 TACCAATACT ATCGCGACTT TGCCGAAAAT AAAGGCAAGT TTGCAGTCGG
201 GCGGAAAGAT ATTGAGTTT ACAACAAAAA AGGGGAGTTG GTCGGCAAAT
251 CAATGACAAA AGCCCCGATG ATTGATTTTT CTGTGGTGTC GCGTAACGGC
301 GTGGCGGCAT TGGTGGGCGA TCAATATATT GTGAGCGTGG CACATAACGG
351 CGGCTATAAC AACGTTGATT TTGGTGCGGA AGGAAGAAAT CCCGATCAAC
401 ATCGTTTAC TTATAAAATT GTGAAACGGA ATAATTATAA AGCAGGGACT
451 AAAGGCCATC CTTATGGCGG CGATTATCAT ATGCCGCGTT TGCATAAATT
501 TGTACAGAT GCAGAACCTG TTGAAATGAC CAGTTATATG GATGGGCGGA

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551 AATATATCGA TCAAAATAAT TACCCTGACC GTGTTTCGTAT TGGGGCAGGC
601 AGGCAATATT GCGCATCTGA TGAAGATGAG CCCAATAACC GCGAAAAGTTC
651 ATATCATATT GCAAGTGCGT ATTCTTGGCT CGTTGGTGCC AATACCTTTG
701 CACAAAATGG ATCAGGTGGT GGCACAGTCA ACTTAGGTAG TGA AAAAATT
751 AAACATAGCC CATATGGTTT TTTACCAACA GGAGGCTCAT TTGGCGACAG
801 TGGCTCACCA ATGTTTATCT ATGATGCCCA AAAGCAAAAG TGGTTAATTA
851 ATGGGGTATT GCAAACGGGC AACCCCTATA TAGGAAAAAG CAATGGCTTC
901 CAGCTGGTTC GTAAAGATTG GTTCTATGAT GAAATCTTTG CTGGAGATAC
951 CCATTCACTA TTCTACGAAC CACGTCAAAA TGGGAAATAC TCTTTTAACG
1001 ACGATAATAA TGGCACAGGA AAAATCAATG CCAAACATGA ACACAATTCT
1051 CTGCCAATAA GATTAAAAAC ACGAACCCTT CAATTGTTTA ATGTTTCTTT
1101 ATCCGAGACA GCAAGAGAAC CTGTTTATCA TGCTGCAGGT GGTGTCAACA
1151 GTTATCGACC CAGACTGAAT AATGGAGAAA ATATTTTCCTT TATTGACGAA
1201 GGAAAAGGCG AATTGATACT TACCAGCAAC ATCAATCAAG GTGCTGGAGG
1251 ATTATATTTT CAAGGAGATT TTACGGTCTC GCCTGAAAAT AACGAAACTT
1301 GGCAAGGCGC GGGCGTTCAT ATCAGTGAAG ACAGTACCGT TACTTGGAAA
1351 GTAAACGGCG TGGCAAACGA CCGCCTGTCC AAAATCGGCA AAGGCACGCT
1401 GCACGTTCAA GCCAAAGGGG AAAACCAAGG CTCGATCAGC GTGGGCGAGC
1451 GTACAGTCAT TTTGGATCAG CAGGCAGACG ATAAAGGCAA AAAACAAGCC
1501 TTTAGTGAAG TCGGCTTGGT CAGCGGCAGG GGTACGGTGC AACTGAATGC
1551 CGATAATCAG TTCAACCCCG ACAAACTCTA TTTTCGGCTTT CGCGGCGGAC
1601 GTTTGGATTT AAACGGGCAT TCGCTTTCGT TCCACCGTAT TCAAAATACC
1651 GATGAAGGGG CGATGATTGT CAACCACAAT CAAGACAAAAG AATCCACCGT
1701 TACCATTACA GGCAATAAAG ATATTGCTAC AACCGGCAAT AACACAGCT
1751 TGGATAGCAA AAAAGAAATT GCCTACAACG GTTGGTTTGG CGAGAAAGAT
1801 ACGACCAAAA CGAACGGGCG GCTCAACCTT GTTTACCAGC CCGCCGAGCA
1851 AGACCGCACC CTGCTGCTTT CCGGCGGAAC AAATTTAAAC GGCAACATCA
1901 CGCAACAAA CGGCAAACTG TTTTCAGCG GCAGACCAAC ACCGCACGCC
1951 TACAATCATT TAAACGACCA TTGGTCGCAA AAAGAGGGCA TTCCTCGCGG
2001 GGAAATCGTG TGGGACAACG ACTGGATCAA CCGCACATTT AAAGCGGAAA
2051 ACTTCCAAAT TAAAGGCGGA CAGGCGGTGG TTTCCCGCAA TGTTGCCAAA
2101 GTGAAAGGCG ATTGGCATTG GAGCAATCAC GCCCAAGCAG TTTTGTGTGT
2151 CGCACCGCAT CAAAGCCACA CAATCTGTAC ACGTTCGGAC TGGACGGGTC
2201 TGACAAATTG TGTCGAAAAA ACCATTACCG ACGATAAAGT GATTGCTTCA
2251 TTGACTAAGA CCGACATCAG CGGCAATGTC GATCTTGCCG ATCAGCTCA
2301 TTTAAATCTC ACAGGGCTTG CCACACTCAA CGGCAATCTT AGTGCAAATG
2351 GCGATACACG TTATACAGTC AGCCACAACG CCACCCAAAA CGGCAACCTT
2401 AGCCTCGTGG GCAATGCCCA AGCAACATTT AATCAAGCCA CATTAAACGG
2451 CAACACATCG GCTTCGGGCA ATGCTTCATT TAATCTAAGC GACCACGCCG
2501 TACAAAACGG CAGTCTGACG CTTTCCGGCA ACGCTAAGGC AAACGTAAGC
2551 CATTCCGCAC TCAACGGTAA TGTCTCCCTA GCCGATAAGG CAGTATTCCA
2601 TTTTGAAAGC AGCCGCTTTA CCGGACAAAT CAGCGGCGGC AAGGATACGG
2651 CATTACACTT AAAAGACAGC GAATGGACGC TGCCGTCAGG CACGGAATTA
2701 GGCAATTAA ACCTTGACAA CGCCACCATT ACACTCAATT CCGCTATCG
2751 CCACGATGCG GCAGGGGCGC AAACCGGCAG TGCGACAGAT GCGCCGCGCC
2801 GCCGTTGCGC CGTTCGCGC CGTTCCTAT TATCCGTTAC ACCGCCAAT
2851 TCGGTAGAAT CCGGTTTCAA CACGCTGACG GTAAACGGCA AATTGAACGG
2901 TCAGGGAACA TTCCGCTTTA TGTGGAACCT CTTGCGCTAC CGCAGCGACA
2951 AATTGAAGCT GGCGAAAAGT TCCGAAGGCA CTTACACCTT GGCGGTCAAC
3001 AATACCGGCA ACGAACCTGC AAGCCTCGAA CAATTGACGG TAGTGGAAGG
3051 AAAAGACAAC AAACCGCTGT CCGAAAACCT TAATTTACC CTGCAAAACG
3101 AACACGTCGA TGCCGCGCGC TGGCGTTACC AACTCATCCG CAAAGACGGC
3151 GAGTTCGCCG TGCATAATCC GGTCAAAGAA CAAGAGCTTT CCGACAAACT
3201 CGGCAAGGCA GAAGCCAAAA AACAGGCGGA AAAAGACAAC GCGCAAGGCC
3251 TTGACGCGCT GATTGCGGCC GGGCGCGATG CCGTCGAAAA GACAGAAAGC
3301 GTTGCCGAAC CGGCCCGGCA GGCAGGCGGG GAAAATGTCG GCATTATGCA
3351 GCGGAGGAA GAGAAAAAAC GGGTGCAGGC GGATAAAGAC ACCGCTTTGG
3401 CGAAACACGC CGAAGCGGAA ACCGCGCCGG CTACCACCGC CTTCCCGCGC
3451 GCGCGCCGCG CCGCGCGGGA TTTGCCGCAA CTGCAACCCC AACCGCAGCC
3501 CCAACCGCAG CGCGACCTGA TCAGCCGTTA TGCCAATAGC GGTTTGAGTG
3551 AATTTTCCGC CACGCTCAAC AGCGTTTTCG CCGTACAGGA CGAATTAGAC
3601 CGCGTATTTG CCGAAGACCG CCGCAACGCC GTTTGGACAA GCGGCATCCG
3651 GGACACCAAA CACTACCGTT CGCAAGATTT CCGCGCCTAC CGCCAACAAA

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3701 CCGACCTGCG CCAAATCGGT ATGCAGAAAA ACCTCGGCAG CGGGCGCGTC
3751 GGCATCCTGT TTTCGCACAA CCGGACCGAA AACACCTTCG ACGACGGCAT
3801 CGGCAACTCG GCACGGCTTG CCCACGGCGC CGTTTTCGGG CAATACGGCA
3851 TCGACAGGTT CTACATCGGC ATCAGCGCGG GCGCGGGTTT TAGCAGCGGC
3901 AGCCTTTCAG ACGGCATCGG AGGCAAAATC CGCCGCCGCG TGCTGCATTA
3951 CGGCATTTCAG GCACGATACC GCGCCGGTTT CGGCGGATTC GGCATCGAAC
4001 CGCACATCGG CGCAACGCGC TATTTCTGTC AAAAAGCGGA TTACCGCTAC
4051 GAAAACGTCA ATATCGCCAC CCCCGGCCTT GCATTCAACC GCTACCGCGC
4101 GGGCATTAAAG GCAGATTATT CATTCAAACC GCGCAACAC ATTTCATCA
4151 CGCCTTATTT GAGCCTGTCC TATACCGATG CCGCTTCGGG CAAAGTCCGA
4201 ACACGCGTCA ATACCGCCGT ATGGGCTCAG GATTTTCGCA AAACCGCAG
4251 TCGGGAATGG GCGGTAAACG CCGAAATCAA AGGTTTCACG CTGTCCCTCC
4301 ACGCTGCCGC CGCCAAAGGC CCGCAACTGG AAGCGCAACA CAGCGCGGGC
4351 ATCAAATTAG GCTACCGCTG GTAA

```

This corresponds to the amino acid sequence <SEQ ID 87; ORF orf1-1>:

orf1-1.pap

```

1 MKTTDKRTE THRKAPKTGR IRFSPAYLAI CLSFGILPOA WAGHTYFGIN
51 YQYYRDFAEK KGKFAVGAKD IEVYNKKGEL VGKSMTKAPM IDFSVVS RNG
101 VAALVGDQYI VSAHNGGYN NVDFGAEGRN PDQHRFTYKI VKRNNYKAGT
151 KGHYPYGGDYH MPRLHKFVTD AEPVEMTSYM DGRKYIDQNN YPDRVRIGAG
201 RQYWRSEDEE PNNRESSYHI ASAYSWLVGG NTFAQNGSGG GTVNLGSEKI
251 KHSPYGFLLPT GGSFGDSGSP MFIYDAQKQK WLINGVLQTG NPYIGKNGF
301 QLVKDKWFYD EIFAGDTHSV FYEPRQNGKY SFNDNNGTG KINAKHEHNS
351 LPNRLKTRTV QLFNVLSET AREPVYHAAG GVNSYRPRLN NGENISFIDE
401 GKGLILTSN INQAGGLYF QGDFTVSPEN NETWQAGGVH ISEDSTVTWK
451 VNGVANDRLS KIGKGLHVQ AKGENQGSIS VGDGTVILDQ QADDKGGKQA
501 FSEIGLVSGR GTVQLNADNQ FNPDKLYFGF RGRRLDLNGH SLSFHRIONT
551 DEGAMIVNHN QDKESTVTIT GNKDIATTGN NNSLDSKKEI AYNGWFGEKD
601 TTKTNGRLNL VYQPAEDRT LLLSGGTNLN GNITQTNGKL FFSGRPTPHA
651 YNHLNDHWSQ KEGIPRGEIV WDNDWINRTF KAENFQIKGG QAVVSRNVAK
701 VKGDWHLNSH AQAVFGVAPH QSHTICTRSD WTGLTNCVEK TITDDKVIAS
751 LTKTDISGNV DLADHAHLNL TGLATLNGNL SANGDTRYTV SHNATQNGNL
801 SLVGNAQATF NQATLNGNTS ASGNASFNLS DHAVQNGSLT LSGNAKANVS
851 HSALNGNVSL ADKAVFHFES SRFTGQISGG KDTALHLKDS EWTLPSTGTEL
901 GNLNLDNATI TLNSAYRHDA AGAQTGSATD APRRRSRRSR RSLLSVTPPT
951 SVESRFNTLT VNGKLNQGT FRFMSELFY RSDKLKLAES SEGTYTLAVN
1001 NTGNEPASLE QLTVEGKDN KPLSENLFNT LQNEHVDAGA WRYQLIRKDG
1051 EFRLHNPVKE QELSDKLGA EAKKQAEKDN AQSLDALIAA GRDAVEKTES
1101 VAEPARQAGG ENVGIMQAE EKKRVQADKD TALAKQREAE TRPATTAFPR
1151 ARRARRDLPO LQPQPQPQPQ RDLISRYANS GLSEFSATLN SVFAVQDELD
1201 RVFAEDRRNA VWTSGIRDIT HYRSQDFRAY RQQTDLRQIG MQKNLGSGRV
1251 GILFSHNRTE NTFDDGIGNS ARLAHGAVFG QYGIDRFYIG ISAGAGFSSG
1301 SLSDGIGGKI RRRVLHYGIQ ARYRAGFGGF GIEPHIGATR YFVQKADYR
1351 ENVNIATPGL AFNRYRAGIK ADYSFKPAQH ISITPYLSLS YTDAAAGKVR
1401 TRVNTAVLAQ DFGKTRSAEW GVNAEIKGFT LSLHAAAAGK PQLEAQSAG
1451 IKLGYRW*

```

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 88>:

orf46-2.seq

```

1 TTGGGCATTT CCCGCAAAAT ATCCCTTATT CTGTCCATAC TGGCAGTGTG
51 CCTGCCGATG CATGCACACG CCTCAGATTT GGCAACGAT TCTTTTATCC
101 GGCAGGTTCT CGACCGTCAG CATTTCGAAC CCGACGGGAA ATACCACCTA
151 TTCGGCAGCA GGGGGGAACT TGCCGAGCGC AGCGGCCATA TCGGATTGGG
201 AAAAATACAA AGCCATCAGT TGGGCAACCT GATGATTCAA CAGCGGGCCA
251 TTAAGGAAA TATCGGCTAC ATTGTCCGCT TTTCCGATCA CGGGCACGAA
301 GTCCATTCCC CCTTCGACAA CCATGCCTCA CATTCCGATT CTGATGAAGC
351 CGGTAGTCCC GTTGACGGAT TTAGCCTTTA CCGCATCCAT TGGGACGGAT

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401  ACGAACACCA TCCCGCCGAC GGCTATGACG GGCCACAGGG CGGCGGCTAT
451  CCCGCTCCCA AAGGCGCGAG GGATATATAC AGCTACGACA TAAAAGGCGT
501  TGCCCAAAAT ATCCGCCTCA ACCTGACCGA CAACCGCAGC ACCGGACAAC
551  GGCTTGCCGA CCGTTTCCAC AATGCCGGTA GTATGCTGAC GCAAGGAGTA
601  GGCGACGGAT TCAAACGCGC CACCCGATAC AGCCCCGAGC TGGACAGATC
651  GGGCAATGCC GCCGAAGCCT TCAACGGCAC TGCAGATATC GTTAAAAACA
701  TCATCGGCGC GGCAGGAGAA ATTGTGCGCG CAGGCGATGC CGTGCAGGGC
751  ATAAGCGAAG GCTCAAACAT TGCTGTCATG CACGGCTTGG GTCTGCTTTC
801  CACCGAAAAC AAGATGGCGC GCATCAACGA TTTGGCAGAT ATGGCGCAAC
851  TCAAAGACTA TGCCGCAGCA GCCATCCGCG ATTGGGCAGT CCAAAACCCC
901  AATGCCGCAC AAGGCATAGA AGCCGTCAGC AATATCTTTA TGGCAGCCAT
951  CCCCATCAAA GGGATTGGAG CTGTTCCGGG AAAATACGGC TTGGGCGGCA
1001 TCACGGCACA TCCTATCAAG CGGTCGCGA TGGGCGCGAT CGCATTGCCG
1051 AAAGGAAAT CCGCGTCAG CGACAATTTT GCCGATGCGG CATACGCCAA
1101 ATACCCGTCC CTTTACCATT CCCGAAATAT CCGTTCAAAC TTGGAGCAGC
1151 GTTACGGCAA AGAAAACATC ACCTCCTCAA CCGTGCCGCC GTCAAACGGC
1201 AAAAAATGTA AACTGGCAGA CCAACGCCAC CCGAAGACAG GCGTACCGTT
1251 TGACGGTAAA GGGTTTCCGA ATTTTGAGAA GCACGTGAAA TATGATACGA
1301 AGCTCGATAT TCAAGAATTA TCGGGGGGCG GTATACCTAA GGCTAAGCCT
1351 GTGTTTGATG CGAAACCGAG ATGGGAGGTT GATAGGAAGC TTAATAAATT
1401 GACAACTCGT GAGCAGGTGG AGAAAAATGT TCAGGAAATA AGGAACGGTA
1451 ATATAAACAG TAACTTTAGC CAACATGCTC AACTAGAGAG GGAAATTAAT
1501 AAATAAAAT CTGCCGATGA AATTAATTTT GCAGATGGAA TGGGAAATTT
1551 TACCGATAGC ATGAATGACA AGGCTTTTAG TAGGCTTGTC AAATCAGTTA
1601 AAGAGAATGG CTTACAAAT CAGTTGTGG AGTACGTGA AATAAATGGA
1651 AAAGCATATA TCGTAAGAGG AAATAATRGG GTTTTGTCTG CAGAATACCT
1701 TGGCAGGATA CATGAATTAA AATTAAAAAA AGTTGACTTT CCTGTTCTTA
1751 ATACTAGTTG GAAAAATCCT ACTGATGTCT TGAATGAATC AGGTAATGTT
1801 AAGAGACCTC GTTATAGGAG TAAATAA

```

This corresponds to the amino acid sequence <SEQ ID 89; ORF orf46-2>:

orf46-2.pep

```

1  LGISRKISLI LSILAVCLPM HAHASDLAND SFIRQVLDRO HFEPDGKYHL
51  FSGRGELAER SGHIGLGKIQ SHQLGNLMIQ QAAIKGNIGY IVRFSDHGHE
101 VHSPFDNHAS HSDSDEAGSP VDGFSLYRIH WDGYEHPAD GYDGPQGGGY
151 PAPKGARDIY SYDIKVAQN IRLNLTNRS TGQRLADRFH NAGSMLTQGV
201 GDGFKRATRY SPELDRSGNA AEAFTGTADI VKNIIGAAGE IVGAGDAVQG
251 ISEGSNIAVM HGLGLLSTEN KMARINDLAD MAQLKDYAAA AIRDWAVQNP
301 NAAQGIEAVS NIFMAAIPK GIGAVRGKYG LGGITAHPIK RSQMGAIALP
351 KGKSAVSDNF ADAAYAKYPS PYHSRNIRSN LEQRYGKENI TSSTVPPSNG
401 KNVKLADQRH PKTGVPFDGK GFPNFEKHVK YDTKLDIQEL SGGGIPKAKP
451 VFDAKPRWEV DRKLNKLTR EQVEKNVQEI RGNINSNFS QHAQLEREIN
501 KLKSADEINF ADGMGKFTDS MNDKAFSRLV KSVKENGFTN PVVEYVEING
551 KAYIVRGNNR VFAAEYLGRI HELKFKKVDF PVPNTSWKNP TDVLNESGNV
601 KRPRYRSK*

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Using the above-described procedures, the following oligonucleotide primers were employed in the polymerase chain reaction (PCR) assay in order to clone the ORFs as indicated:

Oligonucleotides used for PCR

Table 1

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ORF	Primer	Sequence	Restriction sites
279	Forward	CGCGGATCCCATATG-TTGCCTGCAATCACGATT <SEQ ID 90>	BamHI-NdeI
	Reverse	CCCGCTCGAG-TTTAGAAGCGGGCGGCAA <SEQ ID 91>	XhoI
519	Forward	CGCGGATCCCATATG-TTCAAATCCTTTGTCGTCA <SEQ ID 92>	BamHI-NdeI
	Reverse	CCCGCTCGAG-TTTGGCGGTTTTGCTGC <SEQ ID 93>	XhoI
576	Forward	CGCGGATCCCATATG-GCCGCCCCCGCATCT <SEQ ID 94>	BamHI-NdeI
	Reverse	CCCGCTCGAG-ATTTACTTTTTGATGTCGAC <SEQ ID 95>	XhoI
919	Forward	CGCGGATCCCATATG-TGCCAAAGCAAGAGCATC <SEQ ID 96>	BamHI-NdeI
	Reverse	CCCGCTCGAG-CGGGCGGTATTCGGG <SEQ ID 97>	XhoI
121	Forward	CGCGGATCCCATATG-GAAACACAGCTTTACAT <SEQ ID 98>	BamHI-NdeI
	Reverse	CCCGCTCGAG-ATAATAATATCCCGCGCCC <SEQ ID 99>	XhoI
128	Forward	CGCGGATCCCATATG-ACTGACAACGCACT <SEQ ID 100>	BamHI-NdeI
	Reverse	CCCGCTCGAG-GACCGCGTTGTCGAAA <SEQ ID 101>	XhoI
206	Forward	CGCGGATCCCATATG-AAACACCGCCAACCGA <SEQ ID 102>	BamHI-NdeI
	Reverse	CCCGCTCGAG-TTCTGTAAAAAAGTATGTGC <SEQ ID 103>	XhoI
287	Forward	CCGGAATTCTAGCTAGC-CTTTCAGCCTGCGGG <SEQ ID 104>	EcoRI-NheI
	Reverse	CCCGCTCGAG-ATCCTGCTCTTTTTGCC <SEQ ID 105>	XhoI
406	Forward	CGCGGATCCCATATG-TGCGGGACACTGACAG <SEQ ID 106>	BamHI-NdeI
	Reverse	CCCGCTCGAG-AGGTTGTCCTTGTCTATG <SEQ ID 107>	XhoI

EXAMPLE 2

Expression of ORF 919

The primer described in Table 1 for ORF 919 was used to locate and clone ORF 919. The predicted gene *919* was cloned in pET vector and expressed in *E. coli*. The product of

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protein expression and purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 919-His fusion protein purification. Mice were immunized with the purified 919-His and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; PP, purified protein, TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 919 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 919 are provided in Figure 10. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 919 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 3

Expression of ORF 279

The primer described in Table 1 for ORF 279 was used to locate and clone ORF 279. The predicted gene 279 was cloned in pGex vector and expressed in *E. coli*. The product of protein expression and purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 279-GST purification. Mice were immunized with the purified 279-GST and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 279 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 279 are provided in Figure 11. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 279 and the amino acid sequence encoded thereby is provided in Example 1.

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EXAMPLE 4

Expression of ORF 576

The primer described in Table 1 for ORF 576 was used to locate and clone ORF 576. The predicted gene 576 was cloned in pGex vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 576-GST fusion protein purification. Mice were immunized with the purified 576-GST and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B).. These experiments confirm that ORF 576 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipathic regions of ORF 576 are provided in Figure 12. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 576 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 5

Expression of ORF 519

The primer described in Table 1 for ORF 519 was used to locate and clone ORF 519. The predicted gene 519 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 519-His fusion protein purification. Mice were immunized with the purified 519-His and sera were used for Western blot (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 519 is a surface-exposed protein

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and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 519 are provided in Figure 13. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 519 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 6

Expression of ORF 121

The primer described in Table 1 for ORF 121 was used to locate and clone ORF 121. The predicted gene *121* was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 121-His fusion protein purification. Mice were immunized with the purified 121-His and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that 121 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 121 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 121 are provided in Figure 14. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 121 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 7

Expression of ORF 128

The primer described in Table 1 for ORF 128 was used to locate and clone ORF 128. The predicted gene *128* was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 128-His purification. Mice were immunized with the purified 128-His and sera were used for

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Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D) and ELISA assay (panel E). Results show that 128 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 128 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 128 are provided in Figure 15. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 128 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 8

Expression of ORF 206

The primer described in Table 1 for ORF 206 was used to locate and clone ORF 206. The predicted gene 206 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 206-His purification. Mice were immunized with the purified 206-His and sera were used for Western blot analysis (panel B). It is worthnoting that the immunoreactive band in protein extracts from meningococcus is 38 kDa instead of 17 kDa (panel A). To gain information on the nature of this antibody staining we expressed ORF 206 in *E. coli* without the His-tag and including the predicted leader peptide. Western blot analysis on total protein extracts from *E. coli* expressing this native form of the 206 protein showed a recative band at a position of 38 kDa, as observed in meningococcus. We conclude that the 38 kDa band in panel B) is specific and that anti-206 antibodies, likely recognize a multimeric protein complex. In panel C is shown the FACS analysis, in panel D the bactericidal assay, and in panel E) the ELISA assay. Results show that 206 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N. meningitidis* outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 206 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots,

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antigenic index, and amphipatic regions of ORF 519 are provided in Figure 16. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 206 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 9

Expression of ORF 287

The primer described in Table 1 for ORF 287 was used to locate and clone ORF 287. The predicted gene 287 was cloned in pGex vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 287-GST fusion protein purification. Mice were immunized with the purified 287-GST and sera were used for FACS analysis (panel B), bactericidal assay (panel C), and ELISA assay (panel D). Results show that 287 is a surface-exposed protein. Symbols: M1, molecular weight marker. Arrow indicates the position of the main recombinant protein product (A). These experiments confirm that 287 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipatic regions of ORF 287 are provided in Figure 17. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 287 and the amino acid sequence encoded thereby is provided in Example 1.

EXAMPLE 10

Expression of ORF 406

The primer described in Table 1 for ORF 406 was used to locate and clone ORF 406. The predicted gene 406 was cloned in pET vector and expressed in *E. coli*. The product of protein purification was analyzed by SDS-PAGE. In panel A) is shown the analysis of 406-His fusion protein purification. Mice were immunized with the purified 406-His and sera were used for Western blot analysis (panel B), FACS analysis (panel C), bactericidal assay (panel D), and ELISA assay (panel E). Results show that 406 is a surface-exposed protein. Symbols: M1, molecular weight marker; TP, *N. meningitidis* total protein extract; OMV, *N.*

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meningitidis outer membrane vesicle preparation. Arrows indicate the position of the main recombinant protein product (A) and the *N. meningitidis* immunoreactive band (B). These experiments confirm that 406 is a surface-exposed protein and that it is a useful immunogen. The hydrophilicity plots, antigenic index, and amphipathic regions of ORF 406 are provided in Figure 18. The AMPHI program is used to predict putative T-cell epitopes (Gao et al 1989, *J. Immunol* 143:3007; Roberts et al. 1996, *AIDS Res Human Retroviruses* 12:593; Quakyi et al. 1992, *Scand J Immunol Suppl* 11:9). The nucleic acid sequence of ORF 406 and the amino acid sequence encoded thereby is provided in Example 1.

The foregoing examples are intended to illustrate but not to limit the invention.

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Claims

1. A method for identifying an amino acid sequence, comprising the step of searching for putative open reading frames or protein-coding sequences within one or more of *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
2. A method according to claim 1, comprising the steps of searching a *N. meningitidis* nucleotide sequence for an initiation codon and searching the upstream sequence for an in-frame termination codon.
3. A method for producing a protein, comprising the step of expressing a protein comprising an amino acid sequence identified according to any one of claims 1-2.
4. A method for identifying a protein in *N. meningitidis*, comprising the steps of producing a protein according to claim 3, producing an antibody which binds to the protein, and determining whether the antibody recognises a protein produced by *N. meningitidis*.
5. Nucleic acid comprising an open reading frame or protein-coding sequence identified by a method according to any one of claims 1-2.
6. A protein obtained by the method of claim 3.
7. Nucleic acid comprising one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
8. Nucleic acid comprising a nucleotide sequence having greater than 50% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

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9. Nucleic acid comprising a fragment of a nucleotide sequence selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
10. Nucleic acid according to claim 9, wherein the fragment is unique to the genome of *N. meningitidis*.
11. Nucleic acid complementary to the nucleic acid of any one of claims 7-10.
12. A protein comprising an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
13. A protein comprising an amino acid sequences having greater than 50% sequence identity to an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
14. A protein comprising a fragment of an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.
15. Nucleic acid encoding a protein according to any one of claims 6-8.
16. A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to any one of claims 7-11.
17. A computer, a computer memory, a computer storage medium or a computer database containing one or more of the *N. meningitidis* nucleotide sequences selected from the group consisting of SEQ ID NO 1 and the NMB open reading frames.

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18. A polyclonal or monoclonal antibody which binds to a protein according to any one of claims 12-14 or 6.
19. A nucleic acid probe comprising nucleic acid according to any one of claims 5, 7-10, or 15.
20. An amplification primer comprising nucleic acid according to any one of claims 5, 7-10, or 15.
21. A composition comprising (a) nucleic acid according to any one of claims 5, 7-10, or 15; (b) protein according to any one of claims 12-14; and/or (c) an antibody according to claim 18.
22. The use of a composition according to claim 21 as a medicament or as a diagnostic reagent.
23. The use of a composition according to claim 21 in the manufacture of (a) a medicament for treating or preventing infection due to Neisserial bacteria and/or (b) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria.
24. A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 21.

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FIG. 1A

919 (46 kDa)

PURIFICATION

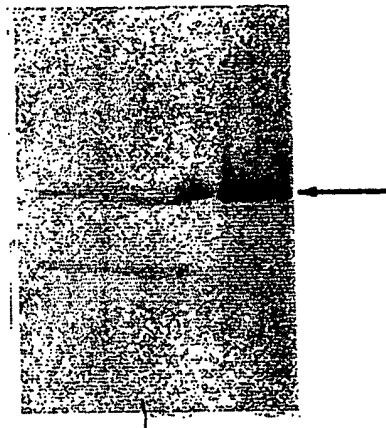
M1 919

*FIG. 1B*

919 (46 kDa)

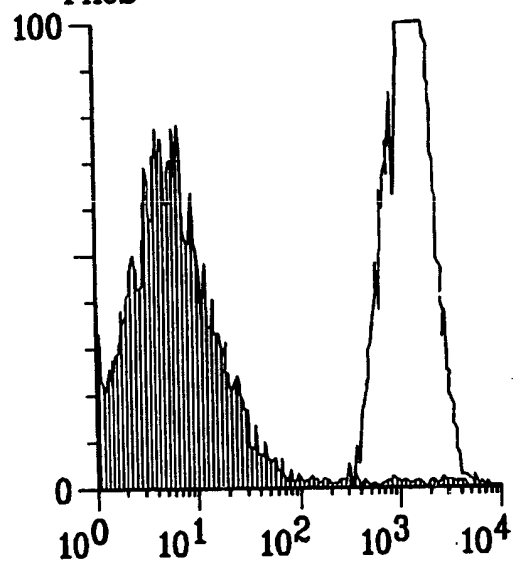
WESTERN BLOT

OMV TP PP

*FIG. 1C*

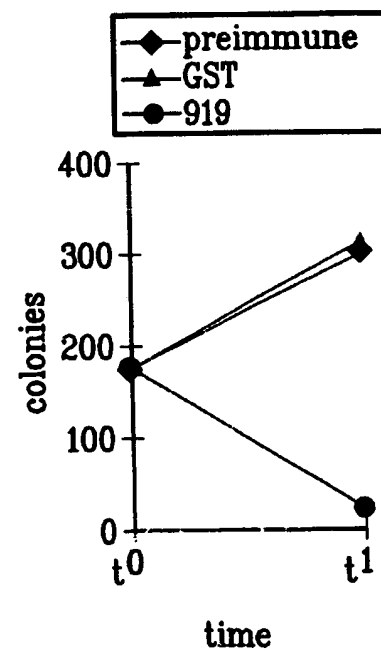
919 (46 kDa)

FACS

*FIG. 1D*

919 (46 kDa)

BACTERICIDAL ASSAY

*FIG. 1E*

919 (46 kDa)

ELISA assay: positive

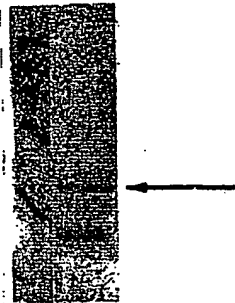
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FIG. 2A

279 (10.5 kDa)

PURIFICATION

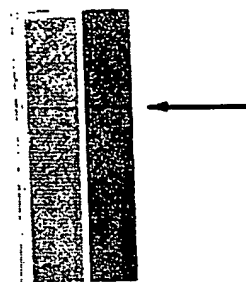
M1 279

*FIG. 2B*

279 (10.5 kDa)

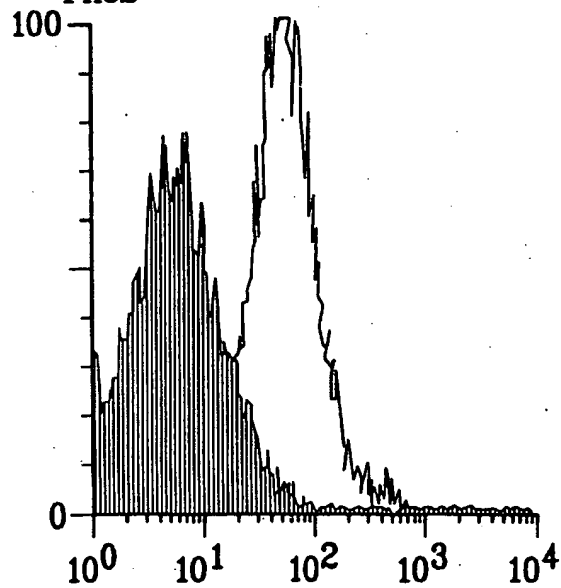
WESTERN BLOT

TP OMV

*FIG. 2C*

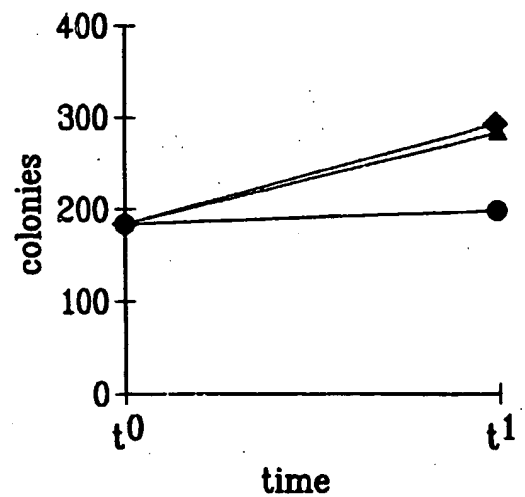
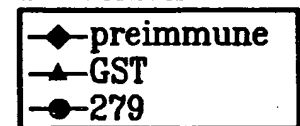
279 (10.5 kDa)

FACS

*FIG. 2D*

279 (10.5 kDa)

BACTERICIDAL ASSAY

*FIG. 2E*

279 (10.5 kDa)

ELISA assay: positive

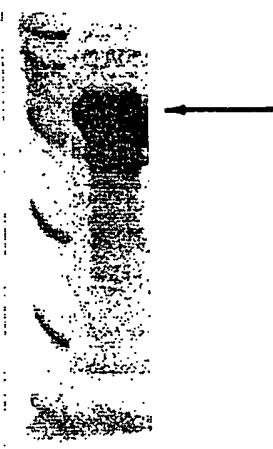
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FIG. 3A

576 (27.8 kDa)

PURIFICATION

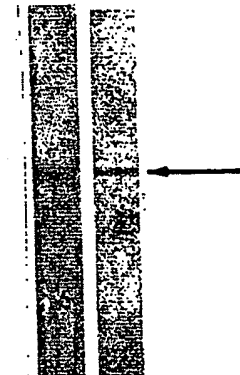
M1 576

*FIG. 3B*

576 (27.8 kDa)

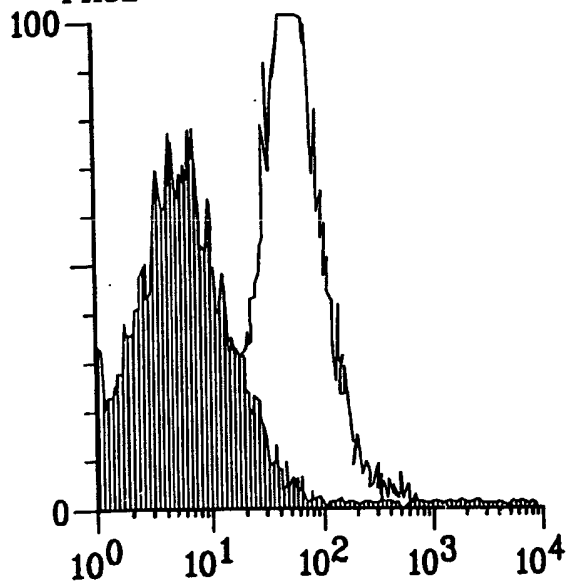
WESTERN BLOT

TP OMV

*FIG. 3C*

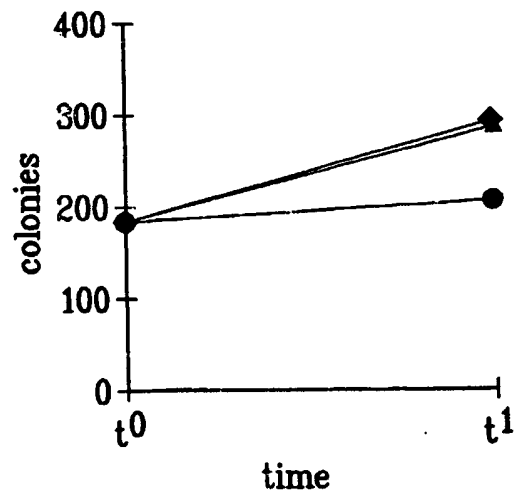
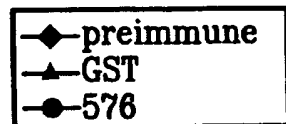
576 (27.8 kDa)

FACS

*FIG. 3D*

576 (27.8 kDa)

BACTERICIDAL ASSAY

*FIG. 3E*

576 (27.8 kDa)

ELISA assay: positive

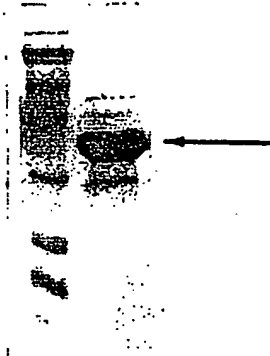
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FIG. 4A

519 (33 kDa)

PURIFICATION

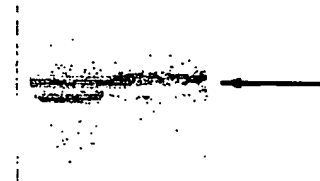
M1 519

*FIG. 4B*

519 (33 kDa)

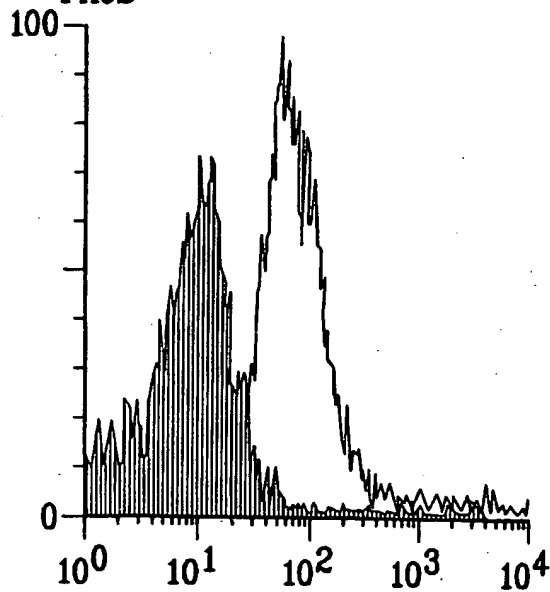
WESTERN BLOT

TP OMV

*FIG. 4C*

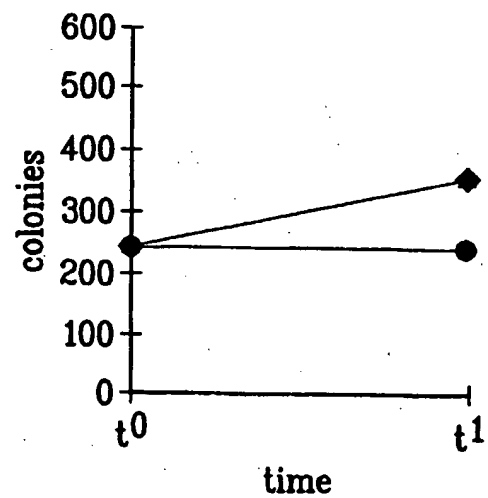
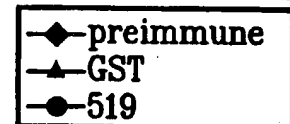
519 (33 kDa)

FACS

*FIG. 4D*

519 (33 kDa)

BACTERICIDAL ASSAY

*FIG. 4E*

519 (33 kDa)

ELISA assay: positive

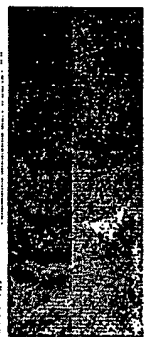
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FIG. 5A

121 (40 kDa)

PURIFICATION

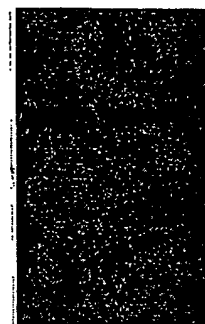
M1 121

*FIG. 5B*

121 (40 kDa)

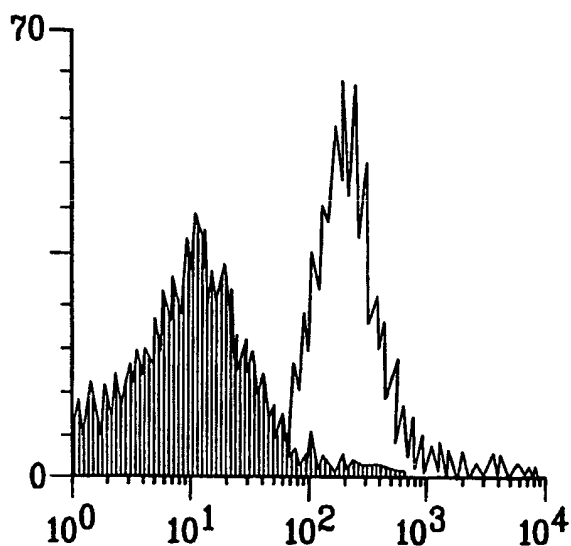
WESTERN BLOT

TP OMV

*FIG. 5C*

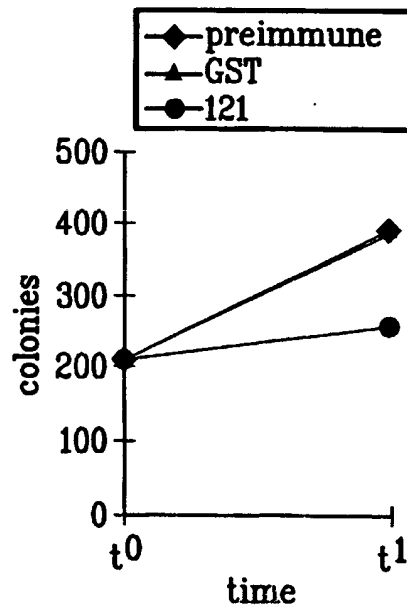
121 (40 kDa)

FACS

*FIG. 5D*

121 (40 kDa)

BACTERICIDAL ASSAY

*FIG. 5E*

121 (40 kDa)

ELISA assay: positive

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FIG. 6A

128 (101 kDa)

PURIFICATION

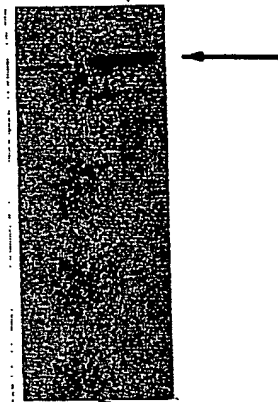
M1 128

*FIG. 6B*

128 (101 kDa)

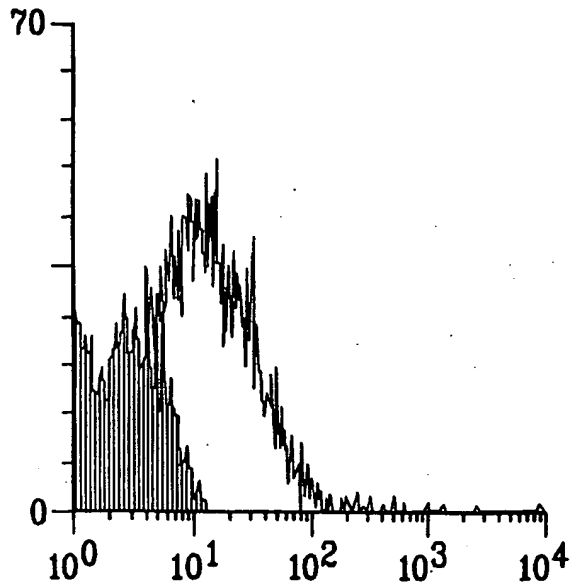
WESTERN BLOT

TP OMV

*FIG. 6C*

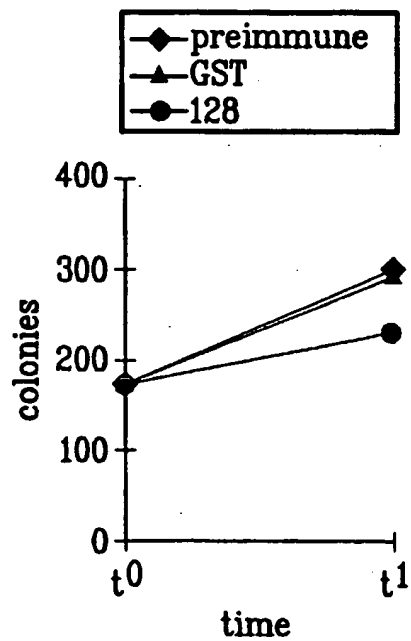
128 (101 kDa)

FACS

*FIG. 6D*

128 (101 kDa)

BACTERICIDAL ASSAY

*FIG. 6E*

128 (101 kDa)

ELISA assay: positive

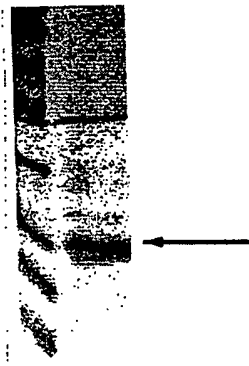
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FIG. 7A

206 (17 kDa)

PURIFICATION

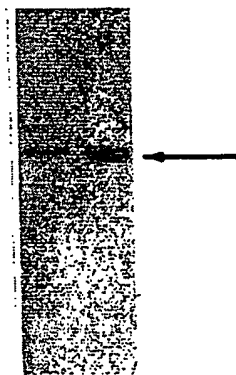
M1 206

*FIG. 7B*

206 (17 kDa)

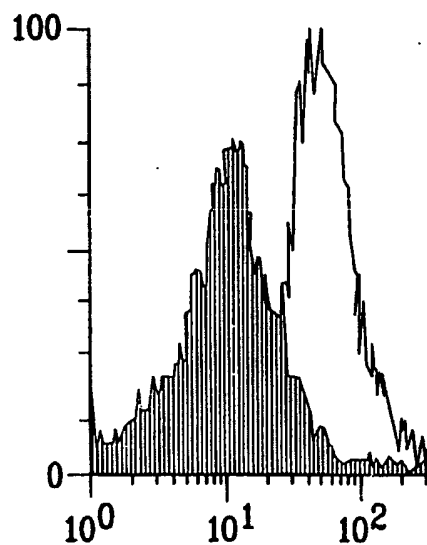
WESTERN BLOT

TP OMV

*FIG. 7C*

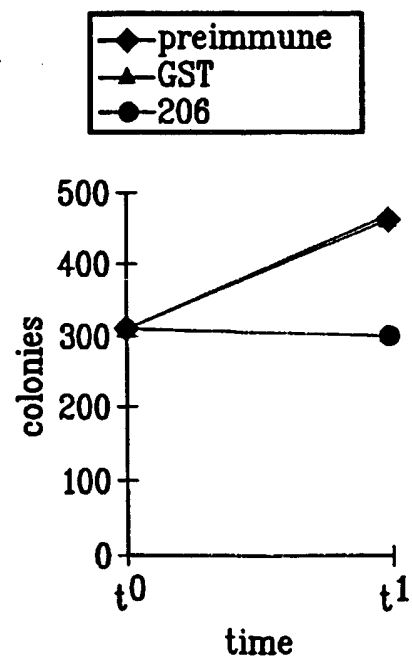
206 (17 kDa)

FACS

*FIG. 7D*

206 (17 kDa)

BACTERICIDAL ASSAY

*FIG. 7E*

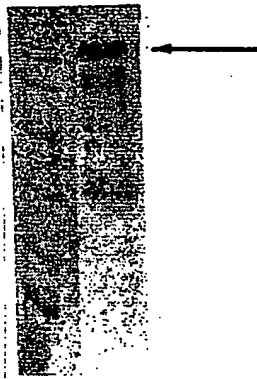
206 (17 kDa)

ELISA assay: positive

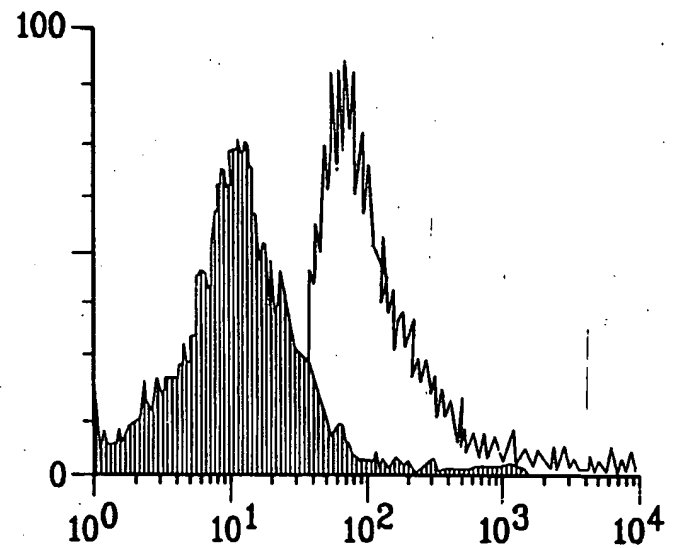
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FIG. 8A

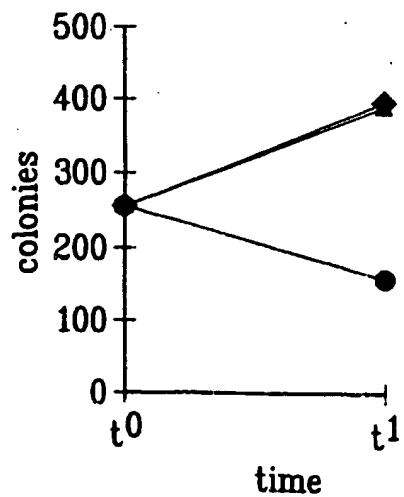
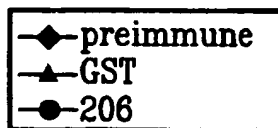
287 (78 kDa)
PURIFICATION
M1 287

*FIG. 8B*

287 (78 kDa)
FACS

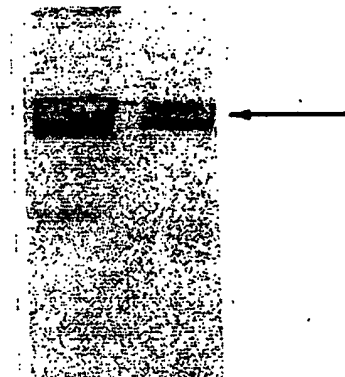
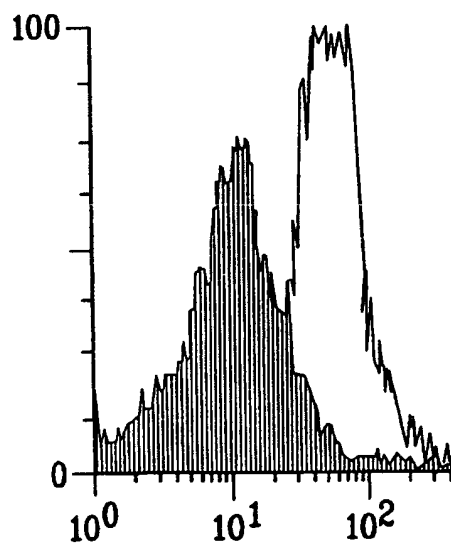
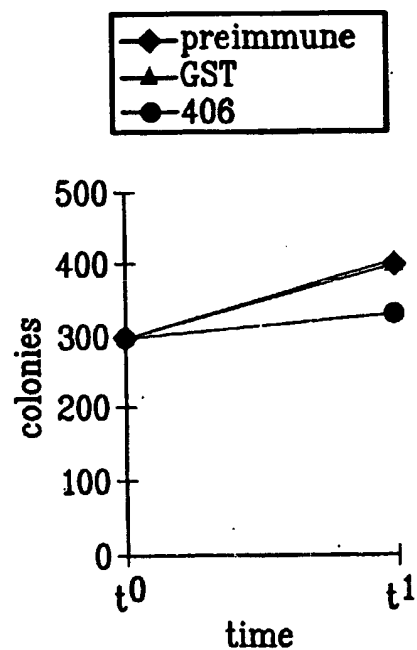
*FIG. 8C*

287 (78 kDa)
BACTERICIDAL ASSAY

*FIG. 8D*

287 (78 kDa)
ELISA assay: positive

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*FIG. 9A*406 (33 kDa)
PURIFICATION
M1 406*FIG. 9B*406 (33 kDa)
WESTERN BLOT
TP OMV*FIG. 9C*406 (33 kDa)
FACS*FIG. 9D*406 (33 kDa)
BACTERICIDAL ASSAY*FIG. 9E*406 (33 kDa)
ELISA assay: positive

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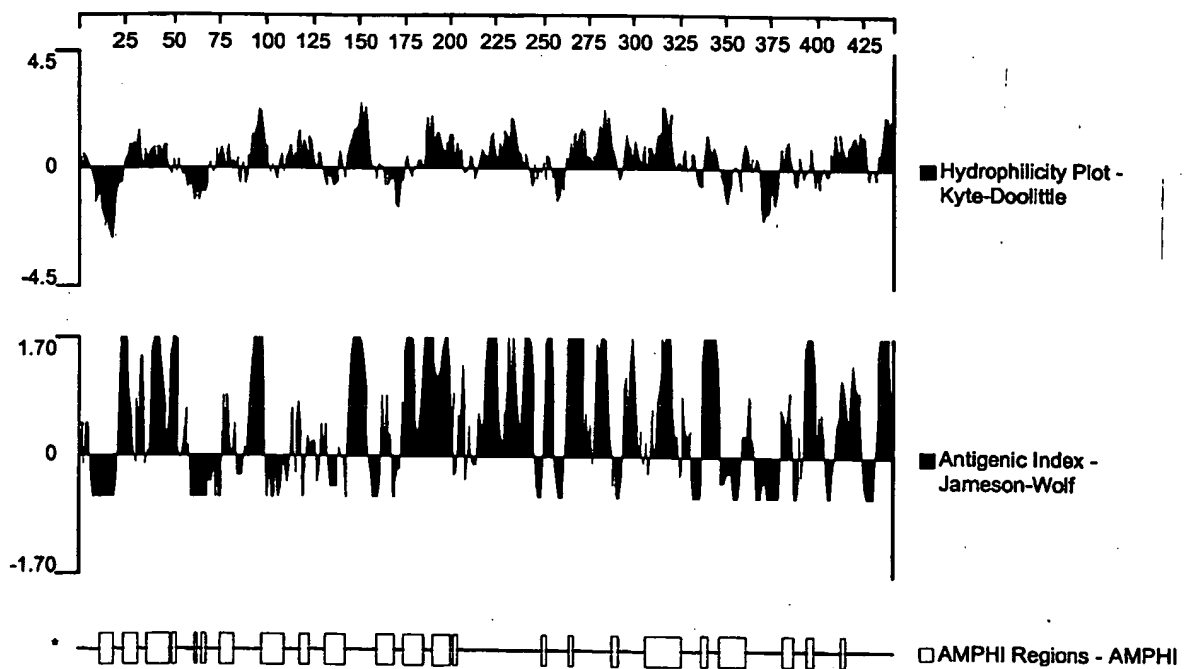
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 10

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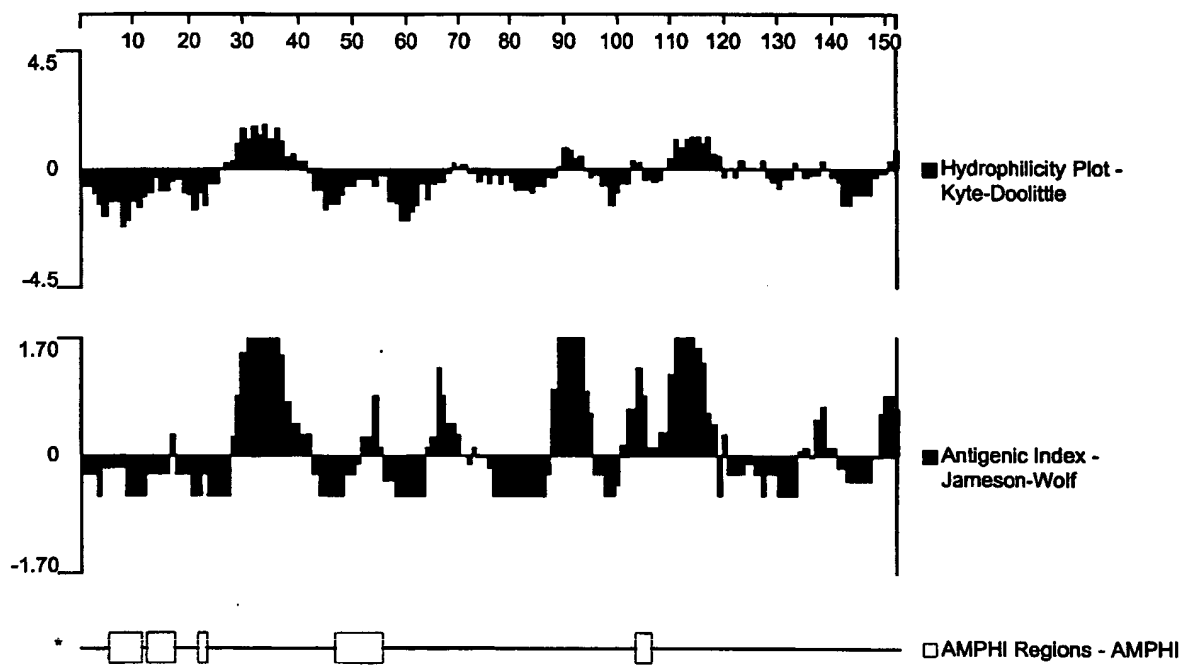
279Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 11

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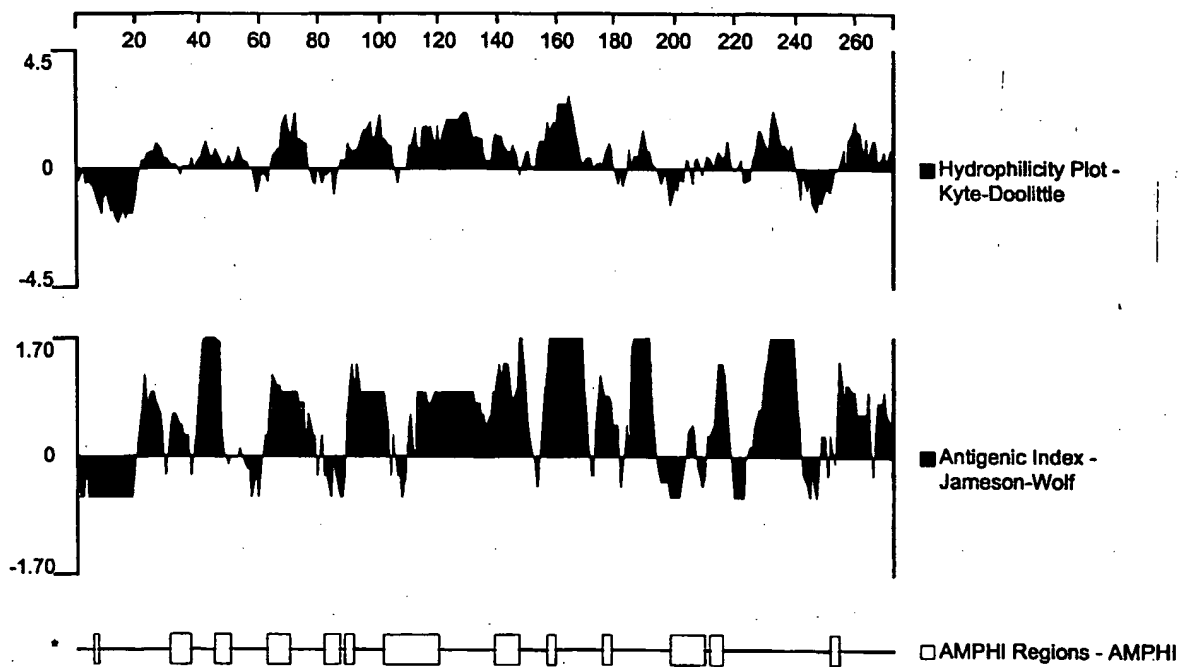
576-1Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 12

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519-1
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

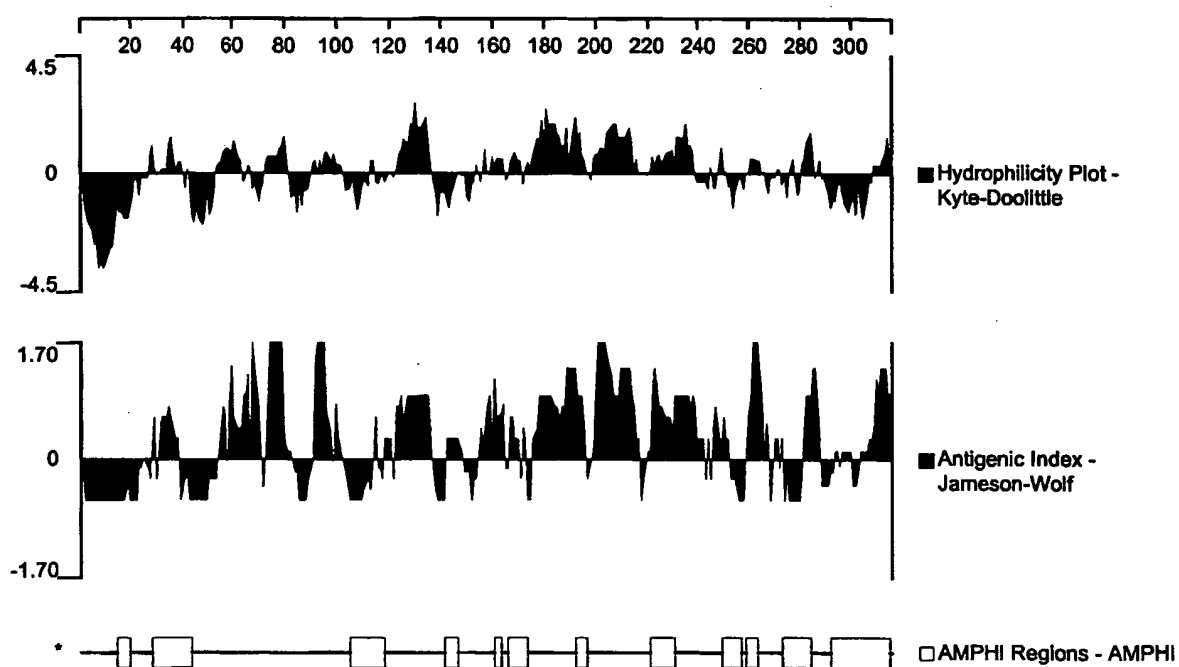


Fig. 13

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121-1
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

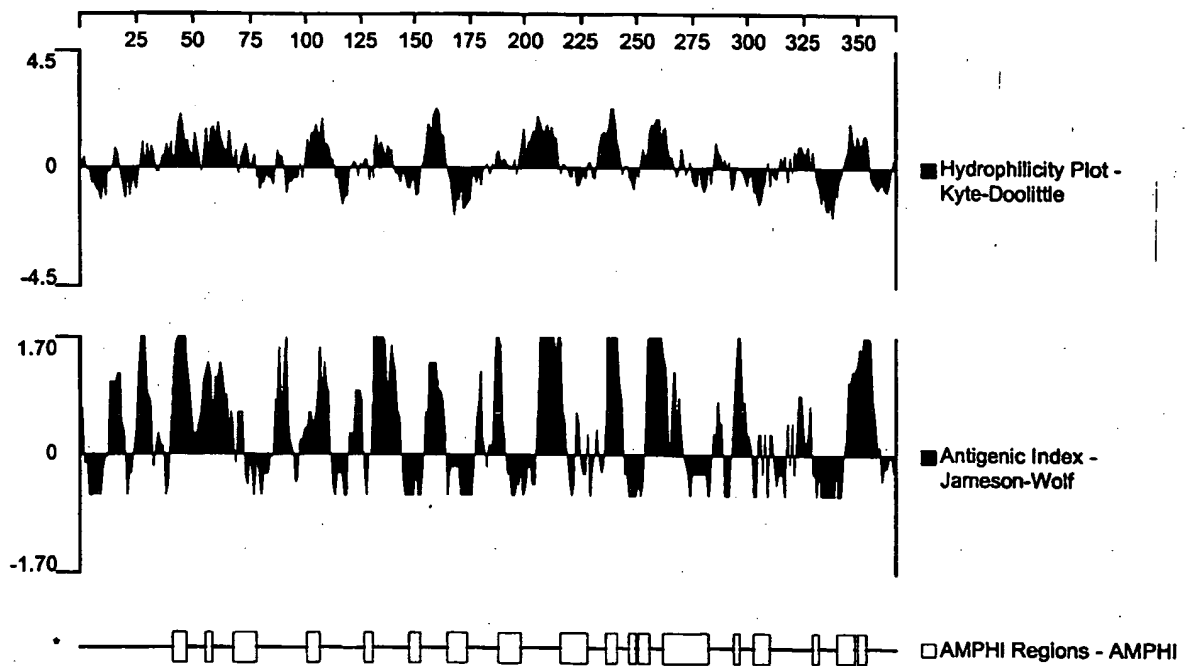


Fig. 14

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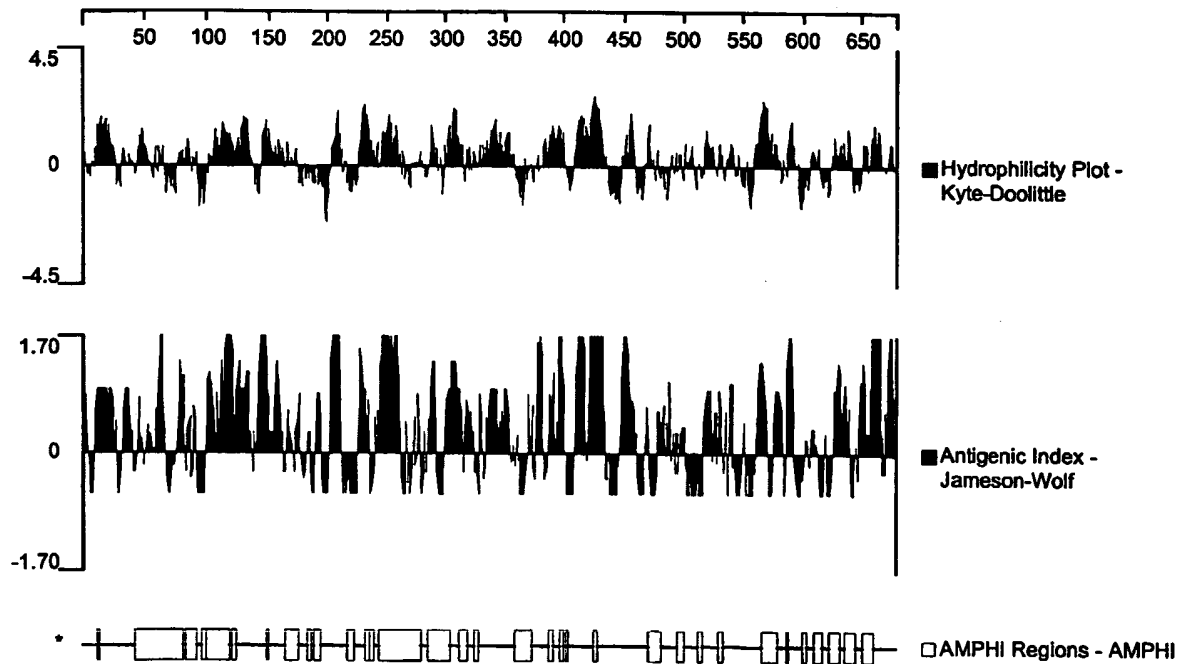
128-1Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 15

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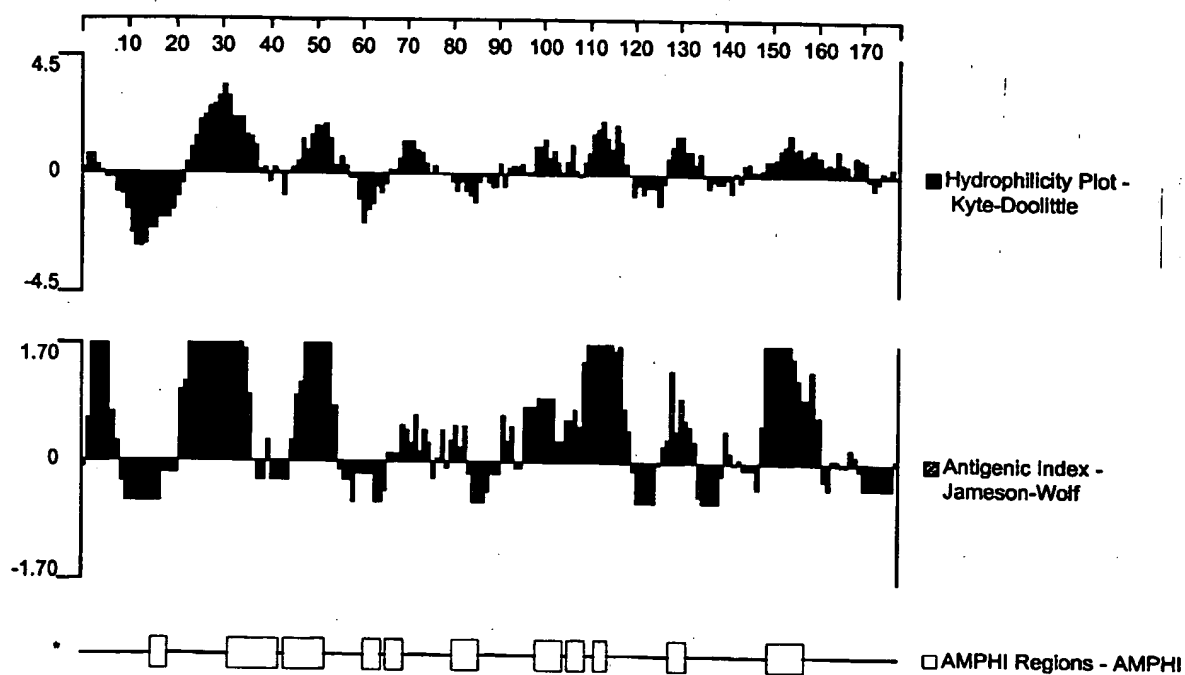
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 16

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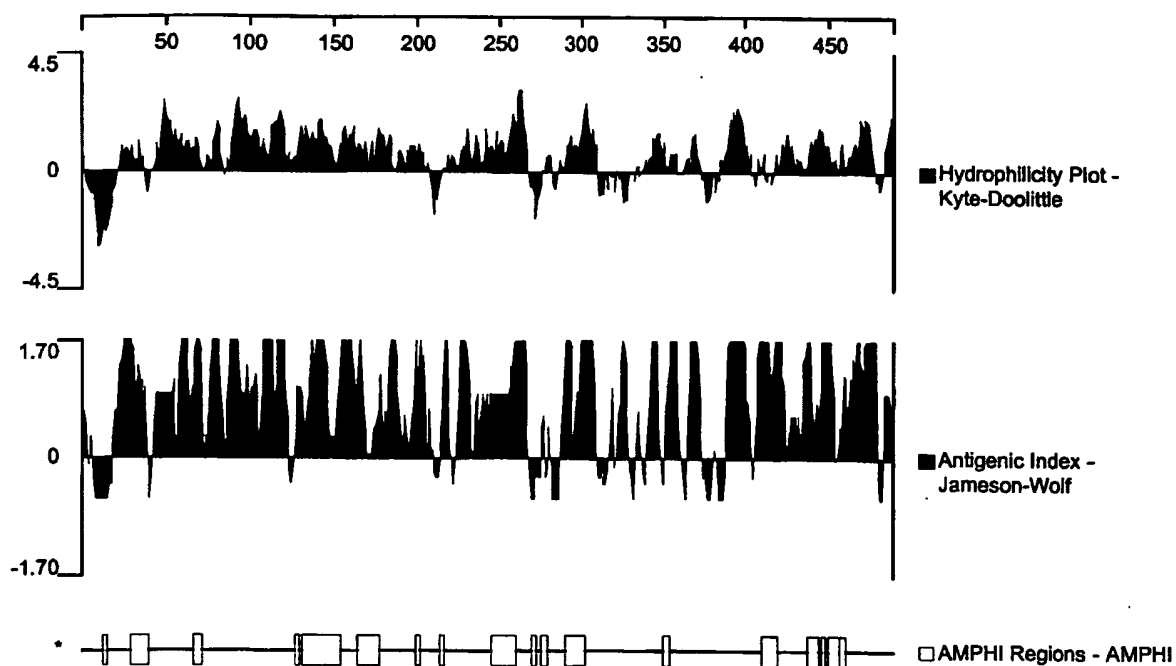
287Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 17

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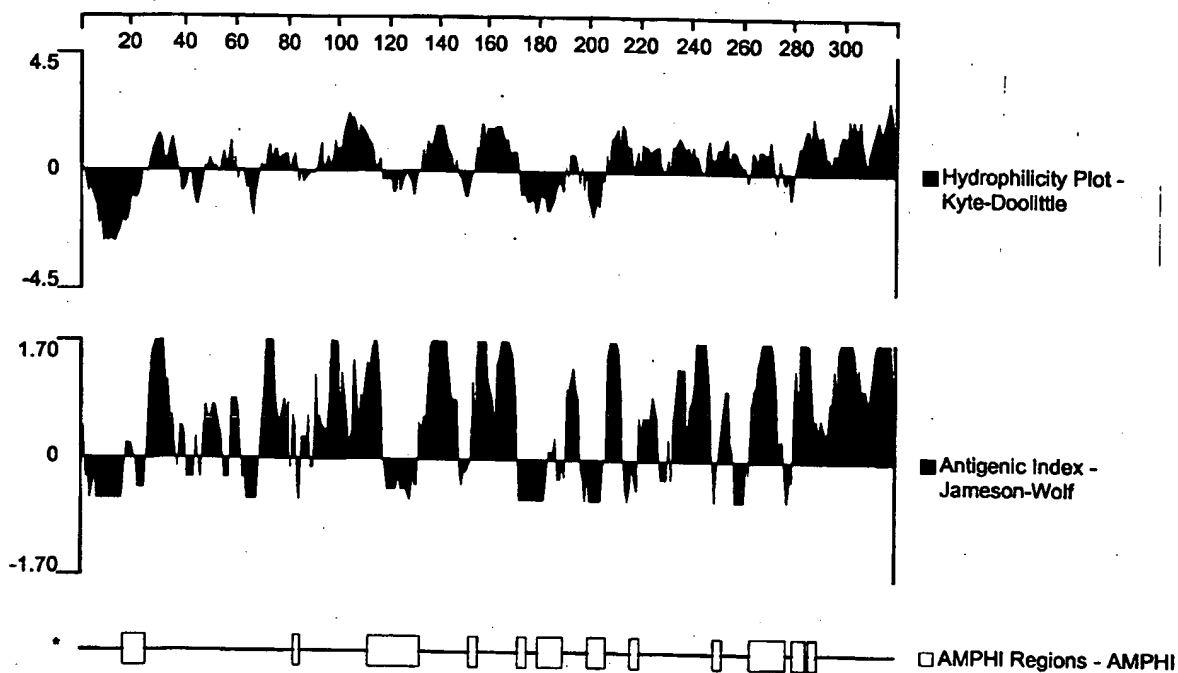
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 18

Appendix A

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APPENDIX A

The following DNA sequence was identified in *N. meningitidis B* <SEQ ID NO. 1>:

TAAACCTTATCCACATCCAAACGCATAACCGTAACCCATTACCGTTATGGAATGTGCGC
CCGACAACCACCCAGCCGAATGATTATATAAATATTTGCACATCAGGCGTATAAAGATAC
AAGAACCTTATCCCGAGCAACGCGCTGCGCTATGCAGTGGGCGACAGCCTCTGCCA
ATGCTTTTCCGCGATATTAGGTAAAACAAAGACATCCCCAACCAATATTATACCGT
GGAAAACCTTCCATATCATGCCGTTGACCGCAGCCGAACCCACAGGATTCCGGAATCA
TCCACAGCCGCAATGCCAGCGGAGTTCGTATCCTTCAAACACCTGCCGTAATAGGCA
TGAATCTTATCCACAGAAGACACGTTCAAATCCGTGCCACTCCTCAAACAACGCTGA
ACCAACCTGCCGATATGCCCGGCTTTCAGCCTGTATGAAAACAGTATTGTCCACAAAG
AGGGAATTCATCGGTCAATTCGCCGACGCTTCGTTCGCCCTGCCGCTAAACCGCATTC
CAAGCATGGTCCAAACGCACTCCGATTGGCTCAAATCTCAGCCTGCCGGGCTTTTGC
GCCATTGCTGCAGGAATTTCCGCTTCCAAACGGGCGATGTCTGCTGAGCCGCTGCAAA
CGCCGGCGCGCATCTTCAAATCCGACTGCATCCCGATGATTTTCCGTCCAGATTGTTT
TGCTTTTGCATAAGCGCGGTAAACGGATTGGATGCTGAGCAGATTGTCTCAGCATCC
CCTGCCCATACGCTTGTAGAAAAACAACCATCAGAAAAATAAATATTTTTCATTTT
AATTTCCATTAAATGCTGTCTGAAGCCGTATTCGACATCAGACGGCATCGCCACGCC
TGTGGATAACTTAAGCGCGGATGCGTTTCAACACTTCTTCTTCCGATTAATGCCAACA
CAGCATCGACGCTGGGGGTTTTCGCGTACCGCAGACGGCAAGGCGCAGGGGCATGCCGA
GTTTGCCCATTTTAAATGCCCTTCTTCTGTCGAGAAGGTTTGAAGAGGTCTGGATGGCT
CGGCATTCCAGTCTTCCAGCCCTTCGAGGCGTTCGGCAAGCGCAGCATACGGGCGCGG
CTTCATCGTCCAGTGTTCGACGCTCTGCTTCGGCAGGCGTTTGTGTGACGTAGAAGT
AGAAGCACTCGTCGGCAAGCGTGTCAAGTCTTGGGGCGGCTTTGACCAAGTCCAACA
CATCTTCCAAAGCAGGTTTTTCGGTTTCATGAATATCGCGCAACGCAAGGCGGGTTGA
CGAGTTCGGCGAGTTTCCGTTGGGTGTGATTTTGTGTGTTTCGCCGTTGATCCAGTAGA
GTTTTTCAAGTCCATACGGCTTGGAGACGGGGAACGCTTTCAAATCAAACCATTCGA
TGAATTGTTCCATTGTGAAGAATTATCGTCGCGCTGCGCCAGCCAAAGCGTGCAGAT
AGTTGAGCATCGCTTCGGGCGAGGATGCCATTGCGCCGAAATCGGTAATGGCAACGGTAT
CGCCGCTGCGTTTGGAGATTTTTTGCTTGTCTTAAAGATCATCGGCAGGTGGCCGT
ATTCCGGCAGGTTCCGCTCGATGGCTTTAAGATGTTGATTGTTTCGGCGTGTGTTC
CATGGTCGTCGCCCGGATACCGTGGGTAAACGCCCATGTCTAGTCTGTACGACAACGC
AGAAGTTGTAGGTCCGCGTACCGTCGGCGCGGGCGATAATCAGGTCATCGAGTCTCGT
TGGGGATGGAGATTTCGCTTTGACCAAGTCTGTCCATTGGGTCAACCGTCCAAGGCG
TTTTGAAACGACAACGGGTTGTACGTCCGACGGGATTTCGGGCGAGGTTTACCTACTT
CCGGACGTCAGCGCGGTGTAAGTCCGCGAGCCTCTTTTTTCGGCTTTCTCAGCATGG
CTTCCAGCTCTTCTTGTGCAATAGCAGTAGTAGGCATGGCTTTTCTAAAAGTTCCG
CAATGACCTCTTGTAGCGGTGCAACCGCGAGTTTGGTAAACGAGCTTGTCCGCGTTGT
CGTAATTGAGACCGCACTTTCATGCCGTCGAGGATGATGTTGACGGATTCCGGCGTAG
AACCGGCCAAGTCGGTGTCTTCAATACGTAATAGGAACTCGCCTTATGATGGCGGGCAA
ACGCCCATGAAACAGGCGGTGCGCACGCCGCGGATGTGAGGTAGCCGGTGGGGCTGG
GGGCGAAACGGGTTTTCAGCGTCATGATGGCTCCGAAATCTTGAAGCGGTTATTTTAC
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CGACCGAATTCCTTCGGGATTGCGTCCAAAAAAGTTCAATGAAACAGCTAATGAAAA
AATCCCGCCCCATTTTCCAAACGGTAGAGGGATAACGCATATCCCTCTTGACGATAA
AGATTTTTTCTTATTTCCCGCATCAAACCGCGTGGTTCGGCGTGGCAGACATATAAAC
GGACACCCAAATCCTCCGCCATTTCCGCCGCCCGCCCAATGGTAGGGATCGCTGACAA
TCACCACGCTGGCAATACCGTTGGCAGCAAAACCGGACGGATGTTGTTAGGTTTTCAT
AAGTGTGCGCGAAGTGTTCAAACAGGATGTTGCGCGCCGGAACCCCTGTTTGAGTG
CGTACCGCGCCGACCTCGGCTTCGGTCATATAGCCTTTTGTGTCGGCTCCCGTAA
ACACGATTTTGCTACCTGCGGCTCTGATAAAGTGCAGTGGCATGGTTGATGCGTTCCG
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CCGGACATACGGCGGCAAAACCTGCCACCCGTCGATAAACCGCCAAACGGATGAGG
CAAACACAGCAAAAGCGGAAAAACACTCAAACAGAAACCGCCAAACAGGTAATAGCGCA
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TCTGCCGACAGCCGCTTACCGTTTGCCAAAAAATCGGACGCTCAACAGGGCGGGATG
ATCGGCGATGGCAGCGCAGCAGCGCGTCATTGTCAAATTTGGGGTTGTCCAAACCAATTC
CTTATACAAATCATCTTTCACGCGCATCATCCGCGCGCGGATGCCAAGCCCAATTTGTT
GAAAATATCCTTCAATTCGGACAAGTCCGGCGCGGTATCCAAATATTGACCACTTCGGC
AGCAATGCCCGGTTCTTCAAATAGGACAAGGCGGACGCGATTGCTGCAACGCGGATT
GTGAAAATTTTGATTTTCAGGATGACATTTCTTGTCTTCGACAATCCCTTATTATC
GGCTTACACAGGGTTTTACTCAATATCCCGCTACAACCGTACCAACGGTTTACAATAC
CCGAATCGACATACAAGGACAAAACGATGAAATACTTGAATCTTGGCGCAATACCCCT
GCCCGCATATTTGCCCGCATACCGCTTCGGCAGACGAAGTGGCCGGATGGAAGACAAC
ACCCCGCAAGGCTGCAATCGCTCAAAGCCCCGTCAGCATCGTCAACCTTTGGGCGACT
TGGTGCGGCGGCTGCCGAAAAGAGATGCTGCCATGTCAAATGGTACAAGCGCAGAAA
AAAGGCAGCGTCGATATGGTCCGATCGCGCTCGACACATCCGACAATATCGGCAACTTC

Appendix A

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CTCAACAACACTCCTGTTTCTACCCGATTGGCGTTACACCGGGGCGAACAGCCGAAAC
TTTATGAAACCTACGGAAACACTGTGCGGTACTGCCCTTTACCGTCGTCGAAGCACCG
AAATGCGGATACAGGCAGACCATTACCGGGGAGGTAAACGAAAAAGCCTGACCGACGCC
GTCAAACCTCGCCCATTTCAAATGCGGTAAACGCGCGGATGCCGTCTGAAGCCGCTTCAGA
TGGCATTTTCTTTTCCACCCGCTGCGGGTGCAAACTTATCCACTATCTAAAAACAGGC
GGAATCTTTATAATCGGCACCTGTCTACCTATTGTTTACAGCGCATATCCCTGCGGACGC
AACCGCCCGAAACGATATGCCGCCCTTCTTACAGGACCTCCTATGATCCGTTTCGAACA
AGTTTCCAAACCTATCCCGGCGGTTTGAAGCCTGAAAAAGCTCAGCTTCCAAATCAA
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ACTGATTTGCGGCATTACCAAGCCGAGCAGGGGCAAAATCCTGTTTAAAGCGGACGACCT
CGGCACATGTCTCGACAACCAATCGGCTTTATGCGCCAACACATCGGCATCGTGTCCA
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CCTGAAGGACGAGAAATGGACGATCCCGTAACCTCTCCGCGGTTGAACAACAACGCT
GTGCATCGCCCGCGCGCTCGTTTACCAGCCCGGCTGCTGATTGCGGACGAACCTCCGC
CAACCTCGACCGCGCTACGCGCTCGATATTATGGAATTGTTCAAACCTTCCACGAAGC
GGGAATACCGTCACTGTTGCCGCACATGACGAAACCTGATGGCGGACTACGGACACCG
CATCTGCGCTCTCGGAAAGGACGACTCGCATGAGCATCATCCACTACCTCTCGTGCAC
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AGCGGGCAAGCGGTGTTGGGCAAACTCAACGAGTCGCGCAAAATCACAATCTATATGGAA
ACCTCCGCCGCAACAAGCGACAGCGATACCGTCCGCGACCTGCTGGCGCGGACAAACGG
CTCGACAACATCCGTTTATCGGCAAGAGACGGTCTGGAAGAATTACAGTCCAATCTT
GACCAAAATCTGATTTCCATGCTTGACGGCAACCCCTGCGGATGTCTTTATCGTTACC
CCGACCCCGGCAACCGCCGCGCTGCTTATCGCGCGCTTACCGAGACATTACCAAACTG
CCTATGGTGAATCCGCGCTATGGATACCGAATGGGTGCAACGCTGTACCAAAATCAAC
GAGTTTATCCGCAAAATTTTGTGTTTCTTCCCTGACGCTGGGGATGGCGTTCGTCTT
GTGCGACACAACACCATCCGCTGCAAACTCTCAGCCGCAAGAGAAATCGAAATCACC
AAACTCTTGGCGCGCGCGCTGCTTATCCGCGCGCTTCTTATCAAGCCATGTGG
CAGAGCATCTTTCCGCGCGCTCAGCTTGGGGCTTTGCGGTGGCTGCTCTGCGGTG
CGCCCATTTGGTCTGATGCCATTTTCAAACCTACGGACTTAATATCGGCTGGCGGTTCTT
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GCAAAATGCGAATATCGAAGTAGAAGCGACGGACACCATTTTTCGCGCTCATCGTT
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GTTTCAGACGGCATTTTATATCAAAACCGCTTACGCGCGCGGTTTTCCAAAGCGGC
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CGCAAAAGATTTGCCGATGGCTTGCCTTCCGCCAACAGGAAGGTGTTTGGATACCGCC
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CACTTTGCTCGCGGCAATTTTATATCAAAACCGCTTACGCGCGCGGCTTTCGAAGGCTTTC
CAAAGCGTCGAGTTCCGCCGCCATCAATACGCGCGCGCAGGCAACGGCGCGGCTTGGG
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GTGCGACAAGAAGCGTAGGCTTTACCCAGTTCCAAATCGTTTCTTCTCGCCTTGTGTT
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ATCAGTGTTTGCGATACGGAACAATGATGCGCTGTAAGGCTTCAGACGGCATCGCAA
CCGATCAGCCGATACGCGCTGATTTTCGCGCGGACGCTGCCGAGTTTTCATA
TTTTATAACCGGATCCAAGTGGTAAATCTGTTGACACGCTTTCGCCCTCGCGCGCGC
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GAAAGCTGTTCCACACCTTGCAAAATGCCGTATTGCCCTCGGTTGTGATGTTCCGCCCC
ATCCGTTTCAACTCGGGGACGTCATAAAGCGTTTCAAATACTGTTTCCACACGCGG
CAGCTTCCCTCCGCCACGGCATTAATGCCATAAACTGCGCTGCATATCCGTGGGGAAG
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Appendix A

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Appendix A

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Appendix A

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TCTGTTTGTCTTGTGTTTTCGCCAATTGCCCTTATTTTGAGCCGTGATTTTATTTTGAAT
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Appendix A

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Appendix A

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GCGGCTGCTTTAAACTCCAGCCCATCGCGGTAATTACGCGCTGATGCGTTCCGCTTCG
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TCGCGGATGGCGGCTTTGTACAGGATGCGGTCCGCTCGCGCGCGTGGCAGCAGTGC
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GAGAGCCTTTATGATCAGGAGCGGCTGGCGGTGCGCGGCTTTGTGCAAGTGGTCAGGCGG
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CATATGGTTCGCTATCTCTGATGTAGATTGGGAAACCCACTGCCGCAACTTCGTGCAAAATC
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GACCTGACCGCAGCAGCAGCTATTGCGCAATATGGCGCTAGGCGTGGGCTATGCCGTATTG
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GAAGTTCTTGAAATGATAGAGTCAAGTGAATAAGTGCATCAGGTGGATGCCTTGCGCATG
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TTTTCGGGCGATGACGCGGAAGAATATTCACCAAAAAACAAATCCACGCTGACAGATGGT
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GGCAAACCTGATACGCAACAATGCGAATACCGATAACAACCAAGCCACCACCACGCAATAC
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ATTGGCTAGCAGACCCTAAAAATAAATGTAATATACAGACTCTAGACTAAACGCGTCTTG
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CAAAACGATGACGGTAAACATCAGATACCTAAATAATCTCCATACCGATCCCTTTCTT
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TAATGCCCAAGCCTTTGAGGCCTTTGGGAGCGTCCGAATATTTTCATTTTTTCGGTAATGC
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Appendix A

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GCGGCTTGGTTTGC GCCCGAAATCATCGCCGGCAAATCGCGCCAATACGGTTTCGGTTCG
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GCGATGCTGCTTGGCTGTTAAACCGCCACGGCGCATCGCTGTTTCGATCAGGATGTG
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CTCGGATGCTTTCAGCTTTCGCAACCGCCCTATGCTGAAAAACCGTGGTTTTCGGC
GAAATCGGCTTAAGCGGCGAAGTCCGCGCGCTCGCACGCGGGAAGAGCGGCTCAAGAA
CGGAAAAACCTCGGCTTCAAAACCGCCATCGTCCCAAGCCAAATATGCCGCGAACGCG
AAAGAGTTTCCGACCTGAAAAATCAGGCGGTTTCGAGTTTTCAGGAAGCCATCGATATT
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GGTTGTGGCGGATTGAAACAAGAAGGCATACCGGCGACAGATAAGATTGCGGCAAGT
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GAAGCGGTTGAAATGCAGAGTGCGGATGCGGCAATCAGGGGAGTATGTGTTTCATCGTA
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CTTCGCGTATGCGCGGATGCGCAGGACGATTTTTCAGCGCGCTGATGCGGACACA
CGCTGACCGCGGATGATTGATTATCCGGTGTTCGATTGGAGGGGTCGGCGCGGAGG
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CGGAAGAGGCGGTAAAGCTCGGTATTCGGATGTTGGCACTGTTCCCGTGGTTACGGCAA
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CAGGTTATCAACCAAGTTTGGAGCAGACGGGCACGCATGCAAAACCGCTATGACGTTACCGTG
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ACCACCAAGCGCGACAAAAACAGCTTCGATTTCAGATGAATTGGGCGCGGTGGCAAT
CATCCGATTAAAGGATTGAAAGACTTTACCGCCACGTTCTGCAAAAAAGCGTATTGCTG
GGCGTTTTGCTGCATTACAGCGTGTTCGATGCGAATGATACGCTGCTGATTATGCGGCCG
TATCAGATTGCCGCCCGCAACGCATTGTGTGAAAAATCAACAGCTCGGCGCAGGCGAAG
AATTGGAGCAAAACCGGAAAGCGGCGCTATGTCTGGCACACACGGGCAGCGGCAAAACG
CTGACCAAGCTTTAAGGCGCGCGCTGTGGCGACGGAATCGGCATTTATCGACAAGGTTTTT
TTCGTGGTGGACAGGAAGGATTGGACTATCAGACGATGAAGGAATACCAACGTTTTTCG
CCGACAGCGTGAACGGTTTCGAAAGCACGGCAGGCTTGAACGCAATTTGAAAAAGAC
GACAAACAAATCATCGTTATCCACCATCCAAAGCTGAACACCTGATGAAGGGCGAAGAT
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TTCGGCGAAGCGCAAAAAACCTGAAAAAGAAATTAATAAATTTCTGCCAGTTCCGGCTT
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CTTTGTGCTTCGCGCGGTGTGCAAAGTCCTGCCGAATTGGACGACAAACTCGCTTCCCTC
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GCCGTGCGTATGAGCGGTTTGGCGGCGATGGGGGCGAAAGTGGATTTCCTTGTGCCAAC
CGCTTTGAAACACGGCTTACGGCTTAACGCCCGAACTTGCCGAAATCGCTGCCGCGCAGGC
GTGGATTTGCTGATTACGGTGGATAACGGTATCGCCAGCATCGCAGGCGTGGCGAGGGCG
CAGGCTCTGGGTTTGGATGTCTATCGTTACTGACCACCACTTGCCGGCCGAGACCGTGGCC
GACTGCATCATCGTCAATCCGAACAAAAAGGCTGCGGTTTCCAAGCAAAAGCTTGGCG
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GGCACGGTGGCGATGTCGTCCCTCTCGACCACAACACCGCATCTCTGTGTCGAAGGT
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- GCTGCTGCGGGTAAACGCTCGGGCAGCAGGTGCTGTGAATTTGCTCTTTAAGCTCTTG
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CAAAATATCGCGGATGACGCCGGCAGGCAGGACTTTTCTCTTTTTCAGGGCGACGCG
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AAACCGTGCTTTCAAGAAAGCTGATTAGGCGGGGTTTTGTGCTCTTTAAGGAAAGTGT
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CGGCATTGCCCAAGAGGGAATGGTTTCAAACATACATTGGGCGATGACCGGCTCCATCA
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GAAAAACGAGTTGTTTGCCGAACACGATGGCCACTTTACACTATACAACGGAATCGATT
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ACAAATAGCGACAACAGGAACCTCCGACAGGGGTTCTTTTGTCTACACCAATAGGCGCTTT
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GGCGGTCTGCGCGCGGTGATTGCCCGCAGCGGAGGACTTGAAAAAGTGAAGGCATC
AGCAAGCGATTGGCGGAACGATTTACAATCATCTGCATTAGCATGTGTCAAGACAAA
ATCCGCTCTGAAAAATATGATACAGCAGGTCCGTATACCGATATATAGTGGATTAAAT

Appendix A

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TAAACCAGTACGGCGTTGCGCTTGCCTTGCCTTACTATTGTACTGTCTGCGGCTTCGTG
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CCTTGTACATGCGCGCATTTGGGTTACGGTAACGTGGCTTTCTGTCGATGAACATGATG

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GCGTTGTTCGGGCGAGGTAGTCCATCAGCGTAGCGGCGGTTTCGCCCTCTTTTTTTCGGGAA
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-TTCTTTGGATCCGGACAGGGCTTGCATGTGCTTTTTTGTGTTCCGCTTCGATTCTT
ATCCCTTCGCGTCTTTTGC CGCAGACTGCTGGATGTGCGCTCTTCTGCTCTTT

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GC GG TTTT TGG CGG TTTT TGG ACT CTT TGC CCT CTT TGG CGC CT CTT TGC CGC CT GT
TTTT TAT CTTT CACT GCG CCA TTTT TGC CTTT TTT CTTT TTT TGC CTT CCG CGC GTT CGGG
CT GT TCT TTTT TCT TCT CGT CT GT TTTT CACT TCG CGG AAC GGT TGT GTG CGC GT C
GTGG CGG CAA CGCG CGG GTG GAAAAA AC GAG CAT CAG GGA AGC AGA AGGG GTT GTA
GCG CAT GGT TCG ACCT TCG GAAAAA GTT GATA AACT GA AGG CTG CAC GAA AGC AG CCG
GAC GTT TGG ATT A TACT GT CAG TTAT GCC GT CTG AAA AT GCC GTT TGC CCA AT CTT GCG C
CTT CTT TGC CGG GATA CTG CAAT CGG CT CAA ACAG CTT ATATT GTG CGT CAT ATTTT C
AAT GCC CAA CGG ATATT GTT GTT CCG ACAC AGGG TAG CAC ATTA AGC CGC CAT ACC GTA
TGT TGC CGG ATTT TGG AAC GTG CGC CCT CCA AAC AA GCA AGC CTG CGC GTT TAC G
GAAA AC GGG GAT CAA CCG GATA AGG AAA TTTT GAT GA ACAG ACT GCT ACT GCT GTG C
GCC GT CTT GCT GACT GCCT GCG CGC AG CGG CAA ACC GATA AAA AT CGG AC GGG CAA GT ACC
GTTT TCA CATA CTG GGC AAAA AC GAC CGT ATCG AAG TGG AAG GATT C GAC GAT CCG GAC
GTT CAA GGG GTT GCT GTT ATATT TCGT ATG CAAAAA AGG CGG CTG AAG GAA ATGGT C
AATT TGA AGAG GAC GCGT CCG AC GCA TCG GTT TCGT GCGT CAG AC GGC ATCT TCG ATT
TCTTT TGA CAA ACC CGC GTG CCG CAA ACC GAA AGA GTT TTT CAA AC CGG TCG GAG CTT C
GCGT TCA AGAG CGG CAG ATT GT CCGT TATT AC GAC CCA AAG CAA ACCT TCG CTT AT
TTGGT GTAC AGC GATA AAA ATCAT CAA AGG CTG CCG CAAAAA TCTT AAG CGG GTT TCC
TGT TCG CGG CGG CAG ATACC GCAA ACC GAT GGG GTG CAA GCG CAGT ACT TCG GCA ACCT G
CTT GCG CGG CCG CTG CAT GATT TTT CAA CCG GATAG AAA ATCT C GAC AAA CGT GAT ATG AA
CCT CT CCA ACC ACTT TCT CAT CGC CAT GCC GAT ATG GAA GAC GCGT TTTT TCA CAAT C
GGT CGT CTAT ATCT GCAA AC CAGT GAAG AC GCG CACT CGG CAT CGCC AT CAA CAA ACC
CTCT CCG ATTAC GATG GAC ATG ATTTT TCG CCA CCG GCA AAAA CAT CCCC ATG CGG AT
GCAG CAC GAG CAG CTG ATG ATG GCG GT CCG GTG CAG GT CAG CCG CCGT TAT GT CGT GCA
TAC CCG CAT CGG CAA CTG GCAA AGC AGT ATCG CGG TTT CAG ACA ATAT CCG CTA ACTT C
TTCC GAG AC GTG ATT GAAA ATATT CAC GCG AAG GTG CCGT TGA CAA AGC CTG ATCAG
CATAG GCTATT CAA GTT GAG CAA AGG CAG CTG CAA CGC GAA CTG CCG CAA ATG CCGT G
GCT GACT GTT CCG CCG CAG CAA CAC ATCT GTT CAG AT CCCC ATG CAA CCGT TAC G
CGC GCA TT CCG CAA ACT CGC ATG CAC CGC CTG CCG CT GTT T CAG GAG CCG GCA ATG C
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CAG AAG GAG CCG CCA ATTAG GGT ATCT CCA TCTT TGA CAC CCGT TAC GCG CAG CAG
ACG ATG AAA GTT CCG CGC AATCG CCA AGCT GGT TCA AGA ATG CAG CCG CCGT TATTTG
TCGT CGG ACTG CCGT GCA TACC GAC GCG CAG CAA CAGT GAA ATG AC GAC CCGT GTC GCG CA
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AAT CCGT GCT CAG CCA AGT GCG CGC GCA AGC CATCTT GCAC GGT TTTT CAG GCG CCGT C
CGG CGG AATGTTT CAA CCG GCGT GAG GTT AAG CCG CCG GTT AAC ACC CTAC CCGT GAAA
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ACG CCG GAG GCG GTT CCG GTT AAG CAA CCA AT CCG CT TCCCCA AAC CCGT AATCT GCG CCG
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GTT CCG CTTT GAG GCG CAA GGT CCG CCGT AACT CAG ATATT GCG GCGT AAT CCG AAG GT
CGCT GATG CAA TCA CATCTT CCG CCGT TCC GCA CAA CTG AAA CTG TCG CAG CCGG
TAC CCG CAG TGT CCG CCGTAT CCG CAG CAA CCG GTG CAG CCG CCA AGCCTAGT CCG GTAA
AGAT CCG CCA AAG CAT CATA CATATCTT GATAG GTG CTG GAG CAG GAG CAGT AGT CCG
CGT GGA AGG AATA AGC GTT TTT CAT CAA AACT CCG CCG CCG CAGT CAG CCG AAG CCG
GGC GCA CTCTG CCG GAA TTTG GTT GAT GTG GTAAA GTT TTT GCG CAG CTGTTGT
AGCT GTT TTTCTT GCG CAG CAGTGT CCG CAGT GACTTCT CCG CAG GTG CCG CCGTATG C
AGAA TCG CCGT CCG GCGTCTT CAG GCG CAG CAGTCTT TAC CCGT AAAA CTCC CAG C
GGC CCG ATTCTG CCA CAG CTG CCG CAG CTG CAC CCG CAG CAG CAG CAG CAG CAG CAG
CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG
TCGG CAT CCA AGTATAA GAC CCG ATG CCGT TGC CTTAAT CAG GCG CCG CCG AATCATCA

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Appendix A

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- AGGACAAGGGGACGAAGCCGAGACAGTACAAATAGTACGGCAAGGGGAGGTAACGCCGT
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- GCTCGTTCCGCGATGCGCGCTTGTGCGAGCAAAACCTCTTAGGCGCGAGCGGATACAAT
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ACCGAACCGACTAGATTCCCGCCTGCGCGGGAATGACGGCTGCAGATGCCCGACGGTCTT
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Appendix A

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Appendix A

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Appendix A

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CCATTCCAAACCGCTCCAATATGCCTCGGGCAGCATACCGGCTTCAGACGGCATATCGTC
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GGTGGATGCAC GAGCCTTGGACAGTGA AAAAATACATAGACGAAGTATTAACCGCTGGAC
ACGGCAAACTCT ACCAAAGGACGCGCAGACACAGCGTCAATCCGACTGAGGGCTACGGCA
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Appendix A

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CCGTTATAATCCGGGGTTGTCCGGGGCGGGTTTTAAGCCGGCATCGTCTTCCCTATTT
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CAAACAGGTTGCGACGCTTTTAAACGCGGCAAAATAATGAAACGGCGGCATTTCGATGCCG
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[illegible]

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Appendix A

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Appendix A

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ATCGCCCGCCAGCCGGTTTTGCTGCTCGCGCAAAAGCTGCTGAGCTGAATCCCAA
TTCACCGCAGACAGCTGCGCGGCTGGAAGATTTCGATTATGTGTGGATAAGTTTTATT

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TTTACGGCGTATTGGATGAAGGCTGGGCGCAATGGTGCGCCCGCCACGGCTCGGCGGC
AAACAAAAAATGGGCGTGTTCGCCACGCGAGCCCCACCGCCCAACCATCTCGGACTC
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TGGCAGGAAAACATCGGCGCGGAAAATTTATCTGCAAAACCAAAAACCTTATCAGCCAA
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AATATTGCAGATTACGAAGTCAGATTTCAAATCGAGGAAAACCGTGAACCGTTATTGAT
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GGTACGCCACTTCACCGCCAAACACACCGACCTTGACCACAAACACCGCCTCATGTGCT
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GACCTACCTGCCCGAAGCCACACCGCGCGCTCAATATGCACCTTCCGCGCTCGAACA
GGGACAGGACATCGTTTCTGCAACCAATCCAACCGGGTCCGCGGTAAGGCTACGG

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CATTGCCGTCGC CAAACTCGCCGGCCTGCCTGTACGCGCATTGAAATCCGCCCAAAGCA
TTTGAACGGACT GGAAAACCAAGCCGCCGCGAACCCTCCCAACTGGATATTTTCAGTAC
CATGCCGTCTGAAAAAGGAGATGAACCGAATGTGGGCAACTTTGTGGATAAAGCAGAGGA
AAAACATTTTGAAGGTATATTGGCAGCAGCCTTGGAAAACTCGATCCCGACAGCCTGAC
CCCGCGCGAAGCATTGTCAGAACTGTACCGTCTGAAAGATTTGTGCAATCCGTATCTTA
ATTTCCGTTGTCGGAACAGCATCAAACCATATGGAAAAATCTGTGGATAAACATTATCTG
ACAGGAAATTTCAAACATAAAAAATGCCGTCCGAACAGCTCAGACGGCATCCGTCCATT
CGGCT

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NMB Open Reading Frames

NMB0001 acetyltransferase, putative 491 3
NMB0002 hypothetical protein 890 498
NMB0003 glutamyl-tRNA synthetase 2305 914
NMB0004 EpiH/GdmH-related protein 3154 2513
NMB0005 arsenate reductase 3504 3154
NMB0006 thioredoxin-related protein 3628 4304
NMB0007 cell division ATP-binding protein FtsE 4304 4951
NMB0008 cell division protein FtsX, putative 4951 5865
NMB0009 Bola/YrbA family protein 5959 6204
NMB0010 phosphoglycerate kinase 7485 6277
NMB0011 UDP-N-acetylglucosamine 1-carboxyvinyltransferase 8819 7569
NMB0012 conserved hypothetical protein 10310 9342
NMB0013 conserved hypothetical protein 10792 10346
NMB0014 3-deoxy-D-manno-octulosonic-acid transferase 12104 10836
NMB0015 6-phosphogluconate dehydrogenase, decarboxylating 13615 12170
NMB0016 hypothetical protein 13911 14144
NMB0017 UDP-3-O-3-hydroxymyristoyl N-acetylglucosamine deacetylase 16137
15217
NMB0018 pilin Pile 17734 17225
NMB0019 pils cassette 18932 18513
NMB0020 pils cassette 19646 19263
NMB0021 pils cassette 20297 19914
NMB0022 pils cassette 21157 20894
NMB0023 pils cassette 21882 21466
NMB0024 pils cassette 22474 22061
NMB0025 large pils cassette 23489 22821
NMB0026 pils cassette 23868 23594
NMB0027 FKBP-type peptidyl-prolyl cis-trans isomerase 24226 23900
NMB0028 hypothetical protein 24522 24307
NMB0029 glycerate dehydrogenase 24644 25594
NMB0030 methionyl-tRNA synthetase 27729 25675
NMB0031 glucosamine--fructose-6-phosphate aminotransferase (isomerizing)
29683 27848
NMB0032 hypothetical protein 29959 30483
NMB0033 membrane-bound lytic murein transglycosylase A, putative 32229
30907
NMB0034 conserved hypothetical protein 32440 33276
NMB0035 conserved hypothetical protein 33276 34439
NMB0036 conserved hypothetical protein 34706 35968
NMB0037 phnA protein 36372 36046
NMB0038 UDP-N-acetylglucosamine pyrophosphorylase 37817 36450
NMB0039 hypothetical protein 38144 37875
NMB0040 hydrolase, putative 38850 38140
NMB0041 ABC transporter, periplasmic solute-binding protein 38909 39907
NMB0042 conserved hypothetical protein 40004 40849
NMB0043 conserved hypothetical protein 40878 41360
NMB0044 peptide methionine sulfoxide reductase 43033 41468
NMB0045 signal recognition particle protein 43179 44441
NMB0046 hypothetical protein 44451 44672
NMB0047 conserved hypothetical protein 45072 45353
NMB0048 conserved hypothetical protein FRAMESHIFT 47969 48109
NMB0049 pilC2 protein FRAMESHIFT 48116 51279
NMB0050 conserved hypothetical protein 55173 53026
NMB0051 twitching motility protein 56685 55462
NMB0052 twitching motility protein PilT 57891 56851
NMB0053 conserved hypothetical protein 58011 58694
NMB0054 hypothetical protein 58697 59101
NMB0055 pyrroline-5-carboxylate reductase 59153 59941

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NMB0056 DnaK suppressor protein 60091 60504
NMB0057 hypothetical protein 66347 66700
NMB0058 hypothetical protein 66731 66885
NMB0059 dnaJ protein 66972 68090
NMB0060 conserved hypothetical protein 68289 70304
NMB0061 dTDP-6-deoxy-L-lyxo-4-hexulose reductase FRAMESHIFT 70923 69924
NMB0062 glucose-1-phosphate thymidyltransferase 71828 70965
NMB0063 dTDP-D-glucose 4,6-dehydratase 72958 71894
NMB0064 UDP-glucose 4-epimerase 74093 73077
NMB0065 hypothetical protein 74476 75399
NMB0066 rRNA adenine N-6-methyltransferase 75687 76418
NMB0067 polysialic acid capsule biosynthesis protein SiaD, truncation
77283 76609
NMB0068 polysialic acid capsule biosynthesis protein SiaC 78416 77370
NMB0069 polysialic acid capsule biosynthesis protein SiaB 79103 78420
NMB0070 polysialic acid capsule biosynthesis protein synX 80240 79110
NMB0071 capsule polysaccharide export outer membrane protein CtrA 80375
81547
NMB0072 capsule polysaccharide export inner-membrane protein CtrB 81565
82725
NMB0073 capsule polysaccharide export inner-membrane protein CtrC 82728
83522
NMB0074 capsule polysaccharide export ATP-binding protein CtrD 83522 84169
NMB0075 transcriptional accessory protein Tex, putative 84236 86506
NMB0076 methyltransferase HphIm(C), FRAMESHIFT 86540 87539
NMB0077 site-specific DNA methylase, truncation 87529 87876
NMB0078 UDP-glucose 4-epimerase, truncation 87922 88575
NMB0079 dTDP-D-glucose 4,6-dehydratase 88694 89758
NMB0080 glucose-1-phosphate thymidyltransferase 89824 90687
NMB0081 dTDP-4-keto-6-deoxy-D-glucose-3,6-epimerase 90729 91280
NMB0082 capsule polysaccharide modification protein LipA 91308 93419
NMB0083 capsule polysaccharide modification protein LipB 93559 94815
NMB0084 conserved hypothetical protein FRAMESHIFT 95185 96587
NMB0085 sodium/glutamate symporter 96808 98019
NMB0086 hypothetical protein 98121 99134
NMB0087 hypothetical protein 99148 99342
NMB0088 outer membrane protein Pl, putative 101170 99773
NMB0089 pyruvate kinase II 102957 101488
NMB0090 IS1016 family transposase, putative FRAMESHIFT 103217 103857
NMB0091 hypothetical protein 104399 104632
NMB0092 hypothetical protein 104629 104853
NMB0093 hypothetical protein 104856 104939
NMB0094 hypothetical protein 105228 105413
NMB0095 hypothetical protein 105423 105572
NMB0096 hypothetical protein 105676 105843
NMB0097 secretion protein, putative POINT MUTATION 105860 107344
NMB0098 ABC transporter, ATP-binding protein FRAMESHIFT 107313 109396
NMB0099 hypothetical protein 109624 109484
NMB0100 hypothetical protein 109770 109627
NMB0101 IS1016 family transposase, putative FRAMESHIFT 109850 110489
NMB0102 hypothetical protein 110608 111123
NMB0103 bacteriocin resistance protein, putative 111896 111405
NMB0104 hypothetical protein 113073 112402
NMB0105 PhnO-related protein 114197 113358
NMB0106 aspartate carbamoyltransferase, catalytic subunit 114436 115353
NMB0107 aspartate carbamoyltransferase, regulatory subunit 115366 115821
NMB0108 hypothetical protein 115889 116551
NMB0109 conserved hypothetical protein 117948 116620
NMB0110 polypeptide deformylase 118018 118518
NMB0111 methionyl-tRNA formyltransferase 118608 119531
NMB0112 16S RNA methyltransferase 119613 120869
NMB0113 hypothetical protein 120892 121431
NMB0114 nitrogen regulation protein NtrY, putative 121434 123551
NMB0115 nitrogen assimilation regulatory protein NtrX 123547 124821

NMB0116 DNA processing chain A 124915 126105
NMB0117 smg protein, putative 126134 126592
NMB0118 DNA topoisomerase I 126667 128970
NMB0119 hypothetical protein 129741 129049
NMB0120 hypothetical protein 130312 129764
NMB0121 conserved hypothetical protein 130431 130805
NMB0122 conserved hypothetical protein 130897 131463
NMB0123 ferredoxin, 4Fe-4S bacterial type 131589 131837
NMB0124 translation elongation factor Tu 132257 133438
NMB0125 preprotein translocase subunit SecE 133638 133913
NMB0126 transcription antitermination protein NusG 133918 134451
NMB0127 50S ribosomal protein L11 134555 134986
NMB0128 50S ribosomal protein L1 134989 135681
NMB0129 hypothetical protein 135753 135893
NMB0130 50S ribosomal protein L10 135914 136411
NMB0131 50S ribosomal protein L7/L12 136472 136840
NMB0132 DNA-directed RNA polymerase, beta subunit FRAMESHIFT 137027 141208
NMB0133 DNA-directed RNA polymerase, beta' subunit 141368 145540
NMB0134 hypothetical protein 145835 146089
NMB0135 conserved hypothetical protein 146089 146235
NMB0136 30S ribosomal protein S12 146417 146785
NMB0137 30S ribosomal protein S7 146906 147373
NMB0138 elongation factor G (EF-G) 147395 149497
NMB0139 translation elongation factor Tu 149586 150767
NMB0140 30S ribosomal protein S10 150788 151096
NMB0141 transposase, truncation 151241 151603
NMB0142 50S ribosomal protein L3 151777 152418
NMB0143 50S ribosomal protein L4 152421 153038
NMB0144 50S ribosomal protein L23 153038 153349
NMB0145 50S ribosomal protein L2 153358 154188
NMB0146 30S ribosomal protein S19 154198 154473
NMB0147 50S ribosomal protein L22 154485 154811
NMB0148 30S ribosomal protein S3 154824 155513
NMB0149 50S ribosomal protein L16 155500 155913
NMB0150 50S ribosomal protein L29 155916 156104
NMB0151 30S ribosomal protein S17 156107 156367
NMB0152 50S ribosomal protein L14 156592 156957
NMB0153 50S ribosomal protein L24 156972 157292
NMB0154 50S ribosomal protein L5 157305 157841
NMB0155 30S ribosomal protein S14 157847 158149
NMB0156 30S ribosomal protein S8 158168 158557
NMB0157 50S ribosomal protein L6 158574 159104
NMB0158 50S ribosomal protein L18 159121 159471
NMB0159 30S ribosomal protein S5 159493 160008
NMB0160 50S ribosomal protein L30 160004 160186
NMB0161 50S ribosomal protein L15 160191 160622
NMB0162 preprotein translocase SecY subunit 160637 161944
NMB0163 translation initiation factor IF-1 161952 162167
NMB0164 50S ribosomal protein L36 162191 162301
NMB0165 30S ribosomal protein S13 162370 162729
NMB0166 30S ribosomal protein S11 162752 163144
NMB0167 30S ribosomal protein S4 163167 163784
NMB0168 DNA-directed RNA polymerase, alpha subunit 163813 164796
NMB0169 50S ribosomal protein L17 164823 165188
NMB0170 septum site-determining protein MinC 165338 166048
NMB0171 septum site-determining protein MinD 166079 166891
NMB0172 cell division topological specificity factor 166898 167158
NMB0173 transcriptional regulator, LysR family 167165 168082
NMB0174 valyl-tRNA synthetase 171252 168418
NMB0175 conserved hypothetical protein 172158 171352
NMB0176 D-amino acid dehydrogenase, small subunit 173595 172342
NMB0177 sodium/alanine symporter, putative 175065 173677
NMB0178 acyl-(acyl-carrier-protein)--UDP-N-acetylglucosamine O-acyltransferase 176198 175425

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NMB0179 (3R)-hydroxymyristoyl-(acyl carrier protein) dehydratase 176734
176288
NMB0180 UDP-3-O-(3-hydroxymyristoyl)-glucosamine N-acyltransferase 177814
176771
NMB0181 outer membrane protein OmpH, putative 178347 177850
NMB0182 outer membrane protein Omp85 180806 178416
NMB0183 conserved hypothetical protein 182203 180866
NMB0184 1-deoxy-D-xylulose 5-phosphate reductoisomerase 183422 182241
NMB0185 phosphatidate cytidyltransferase 184275 183481
NMB0186 undecaprenyl pyrophosphate synthetase 185024 184281
NMB0187 ribosome recycling factor 185637 185083
NMB0188 conserved hypothetical protein 186944 185820
NMB0189 hypothetical protein 187355 187774
NMB0190 glucose inhibited division protein B 187935 188555
NMB0191 ParA family protein 188657 189427
NMB0192 ribonuclease HII 191274 190693
NMB0193 glucose inhibited division protein A 193238 191346
NMB0194 amino acid symporter, putative 194991 193567
NMB0195 pyridoxal phosphate biosynthetic protein PdxA 195133 196137
NMB0196 ribonuclease E 200197 197441
NMB0197 hypothetical protein 200321 200605
NMB0198 ribosomal large subunit pseudouridine synthase C 200690 201679
NMB0199 lipid-A-disaccharide synthase 201730 202899
NMB0200 hypothetical protein 203501 203115
NMB0201 hypothetical protein 203724 204131
NMB0202 hypothetical protein 204152 204322
NMB0203 dihydrodipicolinate reductase 205207 204401
NMB0204 lipoprotein, putative 205594 205220
NMB0205 ferric uptake regulation protein 205813 206244
NMB0206 leucyl/phenylalanyl-tRNA--protein transferase 206317 207039
NMB0207 glyceraldehyde 3-phosphate dehydrogenase 208326 207298
NMB0208 ferredoxin, 4Fe-4S bacterial type 209364 208528
NMB0209 glutathione-regulated potassium-efflux system protein 209513
211486
NMB0210 site-specific DNA methylase, truncation 212082 212401
NMB0211 L-serine dehydratase 214093 212711
NMB0212 DNA gyrase subunit B 216580 214193
NMB0213 hypothetical protein 216736 217719
NMB0214 oligopeptidase A 217810 219843
NMB0215 conserved hypothetical protein 221035 220472
NMB0216 catalase 222945 221434
NMB0217 RNA polymerase sigma-54 factor RpoN, putative 223293 224141
NMB0218 glycosyltransferase 226194 225067
NMB0219 3-oxoacyl-(acyl-carrier-protein) synthase II 227746 226502
NMB0220 acyl carrier protein 228138 227905
NMB0221 dihydroorotate dehydrogenase 228370 229374
NMB0222 hypothetical protein 229540 230010
NMB0223 hypothetical protein 230140 230355
NMB0224 glutamate-ammonia-ligase adenyltransferase 230556 233243
NMB0225 transposase, IS30 family FRAMESHIFT 234513 233551
NMB0226 conserved hypothetical protein 235470 234781
NMB0227 conserved hypothetical protein 236771 235581
NMB0228 conserved hypothetical protein 237637 236903
NMB0229 conserved hypothetical protein FRAMESHIFT 238552 237662
NMB0230 conserved hypothetical protein 239196 238552
NMB0231 hypothetical protein 239356 239255 N
NMB0232 DNA helicase II 239380 241584
NMB0233 hypothetical protein 241663 241761
NMB0234 hypothetical protein 242111 242647
NMB0235 hypothetical protein 243052 242894
NMB0236 hypothetical protein 243168 243063
NMB0237 hypothetical protein 243535 243179
NMB0238 IS1016 family transposase, degenerate 243588 243849
NMB0239 hypothetical protein 244051 244668

NMB0240 hypothetical protein 244694 246142
NMB0241 NADH dehydrogenase I, A subunit 246607 246960
NMB0242 NADH dehydrogenase I, B subunit 246954 247433
NMB0243 NADH dehydrogenase I, C subunit 247449 248039
NMB0244 NADH dehydrogenase I, D subunit 248032 249285
NMB0245 NADH dehydrogenase I, E subunit 249288 249758
NMB0246 NADH dehydrogenase I, F subunit 250151 251449
NMB0247 hypothetical protein 251452 251886
NMB0248 conserved hypothetical protein 252175 252411
NMB0249 NADH dehydrogenase I, G subunit 252726 254984
NMB0250 NADH dehydrogenase I, H subunit 254990 256063
NMB0251 NADH dehydrogenase I, I subunit 256147 256623
NMB0252 hypothetical protein 256657 257361
NMB0253 NADH dehydrogenase I, J subunit 257400 258068
NMB0254 NADH dehydrogenase I, K subunit 258068 258370
NMB0255 cell filamentation protein Fic-related protein 258407 258979
NMB0256 hypothetical protein 259106 259444
NMB0257 NADH dehydrogenase I, L subunit 259496 261517
NMB0258 NADH dehydrogenase I, M subunit 261616 263109
NMB0259 NADH dehydrogenase I, N subunit 263122 264561
NMB0260 hypothetical protein 264612 264995
NMB0261 geranyltranstransferase 265863 265087
NMB0262 exodeoxyribonuclease, small subunit 266188 265967
NMB0263 conserved hypothetical protein 267358 266438
NMB0264 ABC transporter, ATP-binding protein 269219 267366
NMB0265 Holliday junction DNA helicase RuvA 269966 269385
NMB0266 conserved hypothetical protein 270374 270051
NMB0267 conserved hypothetical protein 271155 270439
NMB0268 RNA methyltransferase, TrmH family 271749 271288
NMB0269 competence protein 272539 271817
NMB0270 bioH protein, putative 272538 273284
NMB0271 hypothetical protein 273284 274069
NMB0272 hypothetical protein 274527 274820
NMB0273 hypothetical protein 274861 275283
NMB0274 ATP-dependent DNA helicase RecQ 277728 275431
NMB0275 indole-3-glycerol phosphate synthase 278575 277796
NMB0276 conserved hypothetical protein 279582 278629
NMB0277 virulence factor MviN 281255 279717
NMB0278 thiol:disulfide interchange protein DsbA 281470 282165
NMB0279 conserved hypothetical protein 283229 282228
NMB0280 organic solvent tolerance protein, putative 283431 285704
NMB0281 peptidyl-prolyl cis-trans isomerase 285809 286852
NMB0282 ribonuclease II-related protein 290243 288366
NMB0283 conserved hypothetical protein 290552 291181
NMB0284 adenylosuccinate lyase 291256 292623
NMB0285 O-antigen acetylase FRAMESHIFT 292707 294573
NMB0286 conserved hypothetical protein 295481 294870
NMB0287 probable ATP-dependent helicase DinG 297668 295521
NMB0288 hypothetical protein 297740 297967
NMB0289 deoxyribodipyrimidine photolyase, FRAMESHIFT 299363 298066
NMB0290 transcriptional regulator, putative 300264 299356
NMB0291 conserved hypothetical protein 300372 300767
NMB0292 conserved hypothetical protein 300819 301421
NMB0293 TonB-dependent receptor, putative 301610 303718
NMB0294 thiol:disulfide interchange protein DsbA 303836 304528
NMB0295 signal recognition particle protein 306232 304865
NMB0296 CcsA-related protein 306452 307255
NMB0297 hypothetical protein 307272 307367
NMB0298 hypothetical protein 307401 307583
NMB0299 comEA-related protein 313097 313540
NMB0300 hypothetical protein 313603 313904
NMB0301 Hypothetical protein 313958 314161
NMB0302 IS1016C2 transposase, degenerate 314284 314933
NMB0303 transposase, degenerate 315024 315307

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NMB0304 class 5 outer membrane protein, degenerate 315549 315295
NMB0305 hypothetical protein 315891 315736
NMB0306 hypothetical protein 316061 316252
NMB0307 phospho-2-dehydro-3-deoxyheptonate aldolase, phe-sensitive 316403
317455
NMB0308 dihydrofolate reductase 317526 318011
NMB0309 conserved hypothetical protein 318840 318367
NMB0310 conserved hypothetical protein 319280 318855
NMB0311 hypothetical protein 319392 319634
NMB0312 virulence-associated protein VapA FRAMESHIFT 321089 323177
NMB0313 conserved hypothetical protein 323422 324885
NMB0314 hypothetical protein 326057 325092
NMB0315 conserved hypothetical protein 326135 327424
NMB0316 conserved hypothetical protein 328616 327933
NMB0317 conserved hypothetical protein 329164 328694
NMB0318 fatty acid efflux system protein 329606 330757
NMB0319 fatty acid efflux system protein 330784 332307
NMB0320 hypothetical protein 332373 332519
NMB0321 50S ribosomal protein L28 332560 332790
NMB0322 50S ribosomal protein L33 332825 332977
NMB0323 UbiH family protein 334353 333172
NMB0324 50S ribosomal protein L27 334964 334695
NMB0325 50S ribosomal protein L21 335297 334992
NMB0326 octaprenyl-diphosphate synthase 335521 336492
NMB0327 conserved hypothetical protein FRAMESHIFT 336500 336944
NMB0328 hypothetical protein 336993 337165
NMB0329 type IV pilus assembly protein 337388 339061
NMB0330 conserved hypothetical protein 339358 339152
NMB0331 kinase, putative 339983 339354
NMB0332 type IV prepilin peptidase 340845 339988
NMB0333 pilus assembly protein PilG 342151 340922
NMB0334 glucose-6-phosphate isomerase 342508 344148
NMB0335 2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase
344361 345179
NMB0336 enoyl-(acyl-carrier-protein) reductase 345337 346119
NMB0337 branched-chain amino acid aminotransferase, putative 347364 346369
NMB0338 hypothetical protein 347506 347985
NMB0339 conserved hypothetical protein 347999 349165
NMB0340 lactoylglutathione lyase FRAMESHIFT 349193 349605
NMB0341 tspA protein 352407 349783
NMB0342 intracellular septation protein A 352613 353140
NMB0343 conserved hypothetical protein 353158 353433
NMB0344 BolA/YrbA family protein 353436 353711
NMB0345 cell-binding factor, putative 353763 354626
NMB0346 hypothetical protein 354700 355455
NMB0347 conserved hypothetical protein 355531 356019
NMB0348 conserved hypothetical protein 356053 357060
NMB0349 glutamyl-tRNA synthetase-related protein 358020 357136
NMB0350 hypothetical protein 358760 358311
NMB0351 transaldolase 359966 358914
NMB0352 sugar isomerase, KpsF/GutQ family 360063 361034
NMB0353 conserved hypothetical protein 361255 361788
NMB0354 hypothetical protein 361788 362366
NMB0355 conserved hypothetical protein 362350 362877
NMB0356 ABC transporter, ATP-binding protein 362924 363685
NMB0357 monofunctional biosynthetic peptidoglycan transglycosylase 364858
364160
NMB0358 shikimate 5-dehydrogenase 365670 364864
NMB0359 glutamate--ammonia ligase 365970 367385
NMB0360 AmpG-related protein 367544 368824
NMB0361 conserved hypothetical protein 368824 369096
NMB0362 hypothetical protein 369205 369282
NMB0363 hypothetical protein 369610 369744
NMB0364 FrpC operon protein 370088 370858

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NMB0365 iron-regulated protein FrpC, truncation 370878 371150
NMB0366 hypothetical protein 372373 371243
NMB0367 hypothetical protein 372823 372440
NMB0368 hypothetical protein 373350 372895
NMB0369 hypothetical protein 373720 373334
NMB0370 hypothetical protein 374229 373855
NMB0371 hypothetical protein 374658 374254
NMB0372 hypothetical protein 375341 374667
NMB0373 hypothetical protein 375915 375559
NMB0374 MafB-related protein 377321 375921
NMB0375 mafA protein 378266 377328
NMB0376 hypothetical protein 378379 378266
NMB0377 conserved hypothetical protein 379516 378389
NMB0378 phosphate permease, putative 379807 381378
NMB0379 oxygen-independent coproporphyrinogen III oxidase 383155 381737
NMB0380 transcriptional regulator, Crp/Fnr family 383360 384091
NMB0381 cys regulon transcriptional activator 385157 384210
NMB0382 outer membrane protein class 4 385521 386246
NMB0383 hypothetical protein 386270 386494
NMB0384 hypothetical protein 386773 387066
NMB0385 thiamin-monophosphate kinase 387100 388053
NMB0386 phosphatidylglycerophosphatase A 388049 388531
NMB0387 ABC transporter, ATP-binding protein 390270 388597
NMB0388 sugar transporter, putative 390657 392009
NMB0389 aldose 1-epimerase 392016 393023
NMB0390 maltose phosphorylase 393260 395515
NMB0391 beta-phosphoglucomutase 395531 396193
NMB0392 L-aspartate oxidase 397882 396377
NMB0393 multidrug resistance protein 398266 397934
NMB0394 quinolinate synthetase A 399530 398421
NMB0395 conserved hypothetical protein 399732 400667
NMB0396 nicotinate-nucleotide pyrophosphorylase 400888 401766
NMB0397 hypothetical protein 401797 402081
NMB0398 transcriptional regulator, ArsR family 402176 402454
NMB0399 exodeoxyribonuclease III 402517 403284
NMB0400 transposase, truncated 404230 404799
NMB0401 proline dehydrogenase 409441 405839
NMB0402 sodium/proline symporter 411216 409693
NMB0403 hypothetical protein 411644 411555
NMB0404 conserved hypothetical protein 411699 412016
NMB0405 competence protein ComM 412033 413526
NMB0406 conserved hypothetical protein 413629 414495
NMB0407 thiol:disulfide interchange protein DsbA 414501 415142
NMB0408 bacitracin resistance protein 415178 415996
NMB0409 conserved hypothetical protein 417783 416575
NMB0410 conserved hypothetical protein 418062 418514
NMB0411 conserved hypothetical protein 418514 419497
NMB0412 cell division protein FtsL-related protein 419491 419757
NMB0413 penicillin-binding protein 2 419821 421563
NMB0414 UDP-N-acetylmuramoylalanyl-D-glutamate--2,6-diaminopimelate ligase
421591 423066
NMB0415 conserved hypothetical protein FRAMESHIFT 423092 424736
NMB0416 UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopimelate--D-
alanyl-D-alanyl ligase 424864 426228
NMB0417 hypothetical protein 426234 426407
NMB0418 phospho-N-acetylmuramoyl-pentapeptide-transferase 426657 427736
NMB0419 conserved hypothetical protein 427865 428458
NMB0420 UDP-N-acetylmuramoylalanine--D-glutamate ligase 428545 429879
NMB0421 cell division protein FtsW 430062 431330
NMB0422 UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide)
pyrophosphoryl-undecaprenol N-acetylglucosamine transferase
431337 432401
NMB0423 UDP-N-acetylmuramate--alanine ligase 432559 433965
NMB0424 D-alanine--D-alanine ligase 434081 434992

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NMB0425 cell division protein FtsQ 435006 435710
NMB0426 cell division protein FtsA 435799 437040
NMB0427 cell division protein FtsZ 437162 438337
NMB0428 conserved hypothetical protein 438479 439786
NMB0429 hypothetical protein 440162 440263
NMB0430 carboxyphosphoenolpyruvate phosphonmutase, putative 440412
441287
NMB0431 methylcitrate synthase/citrate synthase 2 441376 442527
NMB0432 conserved hypothetical protein 442683 443468
NMB0433 aconitate hydratase 1 443549 446152
NMB0434 conserved hypothetical protein 446958 448124
NMB0435 acetate kinase 448541 449737
NMB0436 conserved hypothetical protein 450078 450716
NMB0437 conserved hypothetical protein 451289 450849
NMB0438 hypothetical protein 451463 451828
NMB0439 conserved hypothetical protein 451876 453027
NMB0440 prephenate dehydrogenase, putative 453959 453090
NMB0441 nitrilase 454044 454853
NMB0442 opacity protein FRAMESHIFT 455681 454888
NMB0443 transposase, IS30 family 456456 457418
NMB0444 conserved hypothetical protein 457979 458830
NMB0445 bicyclomycin resistance protein, putative 459352 460581
NMB0446 chorismate mutase/prephenate dehydratase 460662 461747
NMB0447 DNA repair protein RecO 461787 462575
NMB0448 pyridoxal phosphate biosynthetic protein PdxJ 462602 463327
NMB0449 hypothetical protein 463482 463703
NMB0450 hypothetical protein 463968 464411
NMB0451 hypothetical protein 464424 465188
NMB0452 holo-(acyl-carrier protein) synthase 465391 465765
NMB0453 mutT protein 465850 466656
NMB0454 hypothetical protein 466652 467071
NMB0455 conserved hypothetical protein 467123 468262
NMB0456 N-acetylmuramoyl-L-alanine amidase 469573 468326
NMB0457 conserved hypothetical protein 470031 469573
NMB0458 glutamate racemase 470233 471042
NMB0459 conserved hypothetical protein 473202 472096
NMB0460 transferrin-binding protein 2 475573 477708
NMB0461 transferrin-binding protein 1 477798 480542
NMB0462 spermidine/putrescine ABC transporter, periplasmic
spermidine/putrescine-binding protein 483195 481819
NMB0463 30S ribosomal protein S20 483261 483521
NMB0464 phospholipase A1, putative 483685 484830
NMB0465 conserved hypothetical protein 484976 485674
NMB0466 aspartyl-tRNA synthetase 485735 487540
NMB0467 hypothetical protein 487694 487975
NMB0468 biosynthetic arginine decarboxylase 488145 490034
NMB0469 agmatinase 490136 491056
NMB0470 C4-dicarboxylate transporter 491257 492720
NMB0471 conserved hypothetical protein 494006 492933
NMB0472 8-amino-7-oxononanoate synthase 494229 495368
NMB0473 conserved hypothetical protein 495381 496025
NMB0474 biotin synthesis protein BioC, putative 496016 496795
NMB0475 hypothetical protein 497063 498451
NMB0476 hypothetical protein 498457 499551
NMB0477 conserved hypothetical protein 499566 500099
NMB0478 hypothetical protein 500104 500745
NMB0479 conserved hypothetical protein 500771 501127
NMB0480 TspB-related protein 502193 501801
NMB0481 hypothetical protein 502509 502180
NMB0482 hypothetical protein 502900 502625
NMB0483 Hypothetical protein 503191 502910
NMB0484 hypothetical protein 503396 503202
NMB0485 hypothetical protein 503691 503404
NMB0486 conserved hypothetical protein FRAMESHIFT 505078 503739

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NMB0487 hypothetical protein 505244 505152
NMB0488 hypothetical protein 505800 505309
NMB0489 hypothetical protein 506682 505804
NMB0490 PspA-related protein 507809 506910
NMB0491 hypothetical protein 508744 508304
NMB0492 hypothetical protein 509383 509063
NMB0493 hemagglutinin/hemolysin-related protein 517494 509386
NMB0494 DNA helicase, truncation 518107 517625
NMB0495 replication protein 519187 518207
NMB0496 hemolysin activator-related protein 519134 520810
NMB0497 hemagglutinin/hemolysin-related protein 520922 526826
NMB0498 hypothetical protein 526836 527342
NMB0499 hypothetical protein 527471 529090
NMB0500 hypothetical protein 529102 529476
NMB0501 hypothetical protein 529757 530128
NMB0502 hypothetical protein 530166 532115
NMB0503 hypothetical protein 532134 532562
NMB0504 hypothetical protein 532780 532992
NMB0506 hypothetical protein 533691 535208
NMB0507 hypothetical protein 535208 535693
NMB0508 hypothetical protein 535883 536152
NMB0509 hypothetical protein 536335 537114
NMB0510 hypothetical protein 537136 537396
NMB0511 hypothetical protein 537506 539425
NMB0512 hypothetical protein 539437 539856
NMB0513 hypothetical protein 539896 540294
NMB0514 hypothetical protein 540420 540656
NMB0515 hypothetical protein 540656 541036
NMB0516 hypothetical protein 541042 541974
NMB0517 hypothetical protein 542172 542020
NMB0518 hypothetical protein 542486 542734
NMB0519 hypothetical protein 542725 542925
NMB0520 hypothetical protein 542931 543107
NMB0521 hypothetical protein 543492 543947
NMB0522 transposase, truncated 543958 544080
NMB0523 ABC transporter, ATP-binding protein, truncation 544162 544441
NMB0524 ribonuclease BN, putative 545691 544474
NMB0525 aluminum resistance protein, putative 546236 546892
NMB0526 hypothetical protein 546923 547438
NMB0527 6-pyruvoyl tetrahydrobiopterin synthase, putative 547448 547867
NMB0528 conserved hypothetical protein 548139 548507
NMB0529 conserved hypothetical protein 548507 549142
NMB0530 glycosyl hydrolase, family 3 550869 549787
NMB0531 conserved hypothetical protein 552446 550929
NMB0532 protease DO 554147 552651
NMB0533 endonuclease III 554914 554288
NMB0534 conserved hypothetical protein 555373 554963
NMB0535 glucose/galactose transporter 555906 557183
NMB0536 Na⁺/H⁺ antiporter 557477 558853
NMB0537 conserved hypothetical protein 559809 558988
NMB0538 conserved hypothetical protein 560326 559820
NMB0539 porphobilinogen deaminase 560445 561377
NMB0540 aspartate aminotransferase 562977 561787
NMB0541 hypothetical protein 563556 563062
NMB0542 hypothetical protein 563672 563872
NMB0543 L-lactate permease, putative 565630 564047
NMB0544 conserved hypothetical protein 566621 565902
NMB0545 conserved hypothetical protein 566870 570352
NMB0546 alcohol dehydrogenase, propanol-preferring 571566 570523
NMB0547 type IV pilin protein 572238 571852
NMB0548 AcrA/AcrE family protein 572464 573639
NMB0549 ABC transporter, ATP-binding protein 573708 575639
NMB0550 thiol:disulfide interchange protein DsbC 576837 576058
NMB0551 primosomal protein n' 576975 579161

NMB0552 hypothetical protein 580284 579214
NMB0553 transposase, putative, POINT MUTATION 581288 580335
NMB0554 dnaK protein 584451 582526
NMB0555 hypothetical protein 584931 584662
NMB0556 repressor protein, putative 585119 585802
NMB0557 conserved hypothetical protein 585937 586272
NMB0558 hypothetical protein 586435 586896
NMB0559 ubiquinone biosynthesis protein AarF 586934 588442
NMB0560 serine acetyltransferase 589620 588805
NMB0561 grpE protein 589804 590379
NMB0562 conserved hypothetical protein 590874 590662
NMB0563 thiamine biosynthesis lipoprotein AppE 591955 590903
NMB0564 Na(+)-translocating NADH-quinone reductase, subunit F 593325
592111
NMB0565 Na(+)-translocating NADH-quinone reductase, subunit E 593932
593342
NMB0566 Na(+)-translocating NADH-quinone reductase, subunit D 594562
593939
NMB0567 Na(+)-translocating NADH-quinone reductase, subunit C 595338
594565
NMB0568 Na(+)-translocating NADH-quinone reductase, subunit B 596563
595334
NMB0569 Na(+)-translocating NADH-quinone reductase, subunit A 597909
596569
NMB0570 hypothetical protein 599680 598262
NMB0571 conserved hypothetical protein 600400 600044
NMB0572 hypothetical protein 601002 600400
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NMB0574 glycine cleavage system T protein 602042 603139
NMB0575 glycine cleavage system H protein 603304 603687
NMB0576 glutamyl-tRNA reductase 603842 605086
NMB0577 NosR-related protein 605365 605934
NMB0578 copper ABC transporter, periplasmic copper-binding protein 605991
607022
NMB0579 copper ABC transporter, ATP-binding protein 607083 607700
NMB0580 protein disulfide isomerase NosL, putative 607842 608333
NMB0581 electron transfer flavoprotein-ubiquinone oxidoreductase 610085
608427
NMB0582 bacteriocin resistance protein, putative 610757 610218
NMB0583 IS1016C2 transposase 612651 611986
NMB0584 FrpC operon protein 613242 614054
NMB0585 iron-regulated protein FrpA, putative 614074 617979
NMB0586 adhesin, putative 619176 618265
NMB0587 membrane protein 620128 619256
NMB0588 ABC transporter, ATP-binding protein 620907 620155
NMB0589 50s ribosomal protein L19 621563 621201
NMB0590 tRNA (guanine-N1)-methyltransferase FRAMESHIFT 622329 621582
NMB0591 16S rRNA processing protein RimM 622838 622332
NMB0592 30S ribosomal protein S16 623099 622857
NMB0593 conserved hypothetical protein 625570 623147
NMB0594 sensor histidine kinase 627094 625691
NMB0595 DNA-binding response regulator 627785 627111
NMB0596 hypothetical protein 629789 627978
NMB0597 hypothetical protein 630132 629782
NMB0598 Maf/YceF/YhdE family protein 630749 630144
NMB0599 conserved hypothetical protein 631572 630805
NMB0600 hypothetical protein 632272 631589
NMB0601 conserved hypothetical protein 632479 632279
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NMB0605 histone deacetylase family protein 636107 635001
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NMB0608 protein-export membrane protein SecF 638570 639502
NMB0609 30s ribosomal protein S15 639728 639994
NMB0610 spermidine/putrescine ABC transporter, ATP-binding protein 640243
641499
NMB0611 spermidine/putrescine ABC transporter, permease protein 641518
642480
NMB0612 spermidine/putrescine ABC transporter, permease protein 642483
643367
NMB0613 hypothetical protein 643392 643496
NMB0614 oxidoreductase, putative 643496 644788
NMB0615 ammonium transporter AmtB, putative 646340 645039
NMB0616 IS1016 family transposase, degenerate 647272 646871
NMB0617 transcription termination factor Rho 648837 647581
NMB0618 phosphoenolpyruvate synthase 651441 649060
NMB0619 conserved hypothetical protein 651853 652671
NMB0620 phosphoglycolate phosphatase 653575 652916
NMB0621 conserved hypothetical protein 654440 653616
NMB0622 outer membrane lipoprotein carrier protein 654867 655487
NMB0623 spermidine/putrescine ABC transporter, periplasmic
spermidine/putrescine-binding protein 655763 656899
NMB0624 galactosyltransferase-related protein FRAMESHIFT 657035 658253
NMB0625 conserved hypothetical protein 658297 658824
NMB0626 peptide chain release factor 3 660797 659205
NMB0627 phosphoribosyl-AMP cyclohydrolase 661299 660907
NMB0628 hisF protein 662097 661333
NMB0629 phosphoribosylformimino-5-aminoimidazole carboxamide ribotide
isomerase 662847 662113
NMB0630 amidotransferase HisH 663518 662883
NMB0631 phosphate acetyltransferase Pta, putative 665151 663652
NMB0632 iron(III) ABC transporter, ATP-binding protein 666394 665339
NMB0633 iron(III) ABC transporter, permease protein 667932 666418
NMB0634 iron(III) ABC transporter, periplasmic binding protein 668995
668003
NMB0635 transposase, IS30 family 670247 669285
NMB0636 hypothetical protein 670794 670414
NMB0637 argininosuccinate lyase 672228 670855
NMB0638 UTP--glucose-1-phosphate uridylyltransferase 673116 672250
NMB0639 conserved hypothetical protein 673743 673147
NMB0640 hypothetical protein 673969 673739
NMB0641 inorganic pyrophosphatase 674610 674080
NMB0642 dATP pyrophosphohydrolase 675169 674714
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NMB0644 hypothetical protein 677443 677904
NMB0645 ribonuclease FRAMESHIFT 677948 678275
NMB0646 ribonuclease inhibitor barstar 678290 678574
NMB0647 hypothetical protein 679091 680326
NMB0648 hypothetical protein 680357 680776
NMB0649 hypothetical protein 680970 681191
NMB0650 hypothetical protein 681167 681583
NMB0651 hypothetical protein 681687 682073
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NMB0653 MafB-related protein 683144 684409
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NMB0657 hypothetical protein 686024 686224
NMB0658 Hypothetical protein 686055 686312
NMB0659 hypothetical protein 686346 686744
NMB0660 hypothetical protein 686929 687315
NMB0661 bis(5'-nucleosyl)-tetraphosphatase, symmetrical/Trk system
potassium uptake protein TrkG FRAMESHIFT 689659 687362
NMB0662 ribonuclease, putative 690126 689740
NMB0663 outer membrane protein NsgA 690786 690265
NMB0664 hypothetical protein 691151 690960

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NMB0665 oxygen-independent coprophorphyrinogen III oxidase family protein
692546 691374
NMB0666 DNA ligase 695128 692606
NMB0667 hypothetical protein 696562 695279
NMB0668 ampD protein 697352 696783
NMB0669 conserved hypothetical protein 697436 698428
NMB0670 thymidylate kinase 698491 699108
NMB0671 malate oxidoreductase (NAD) 699333 700610
NMB0672 tetraacyldisaccharide 4'-kinase 701160 702191
NMB0673 hypothetical protein 702394 702978
NMB0674 conserved hypothetical protein 703050 703229
NMB0675 3-deoxy-D-manno-octulosonate cytidyltransferase 703229 703987
NMB0676 hypothetical protein 704013 704411
NMB0677 hypothetical protein 704610 704723
NMB0678 tryptophan synthase, alpha subunit 705306 706088
NMB0679 acetyl-CoA carboxylase, carboxyl transferase beta subunit 706129
706998
NMB0680 cryptic protein 707672 707064
NMB0681 conserved hypothetical protein 707781 708002
NMB0682 dihydroorotase 708368 709399
NMB0683 N utilization substance protein B 710195 709773
NMB0684 riboflavin synthase, beta subunit 710749 710276
NMB0685 hypothetical protein 711120 710800
NMB0686 ribonuclease III 711287 712003
NMB0687 GTP-binding protein Era 712003 712974
NMB0688 N-(5'-phosphoribosyl)anthranilate isomerase 715446 714823
NMB0689 transcription elongation factor GreB 715996 715508
NMB0690 amidophosphoribosyltransferase 717640 716099
NMB0691 colicin V production protein, putative 718450 717956
NMB0692 tpc protein 719441 718446
NMB0693 folypolyglutamate synthase/dihydrofolate synthase 720728 719457
NMB0694 folI protein 721205 720762
NMB0695 hypothetical protein 721569 721213
NMB0696 amino acid ABC transporter, ATP-binding protein FRAMESHIFT 722369
721645
NMB0697 dimethyladenosine transferase 723321 722545
NMB0698 hypothetical protein 723518 724204
NMB0699 tryptophan synthase, beta subunit 724290 725489
NMB0700 IgA-specific serine endopeptidase 731118 725674
NMB0701 hypothetical protein 731531 731280
NMB0702 competence protein ComA 732529 734601
NMB0703 competence lipoprotein ComL 735635 734835
NMB0704 ribosomal large subunit pseudouridine synthase D 735634 736755
NMB0705 transporter 737858 736914
NMB0706 conserved hypothetical protein 738418 739194
NMB0707 rare lipoprotein B, putative 739249 739725
NMB0708 DNA polymerase III, delta subunit 739730 740725
NMB0709 Hypothetical protein 740849 741265
NMB0710 Hypothetical protein 741293 741856
NMB0711 conserved hypothetical protein FRAMESHIFT 742826 741946
NMB0712 RNA polymerase sigma-32 factor 744182 743313
NMB0713 apolipoprotein N-acyltransferase, putative 746012 744441
NMB0714 conserved hypothetical protein FRAMESHIFT 746771 746019
NMB0715 Hypothetical protein 746967 747284
NMB0716 Hypothetical protein 747440 747727
NMB0717 cytochrome, putative 748209 747796
NMB0718 ferrochelatase 749572 748493
NMB0719 queuine tRNA-ribosyltransferase 750697 749585
NMB0720 threonyl-tRNA synthetase 751005 752915
NMB0721 translation initiation factor 3 752990 753454
NMB0722 50S ribosomal protein L35 753604 753798
NMB0723 50S ribosomal protein L20 753814 754170
NMB0724 phenylalanyl-tRNA synthetase, alpha chain 754519 755508
NMB0725 modification methylase HgaI-1 755694 756749

NMB0726 type II restriction enzyme HgaI 756755 758221
NMB0727 N-6 adenine-specific DNA methylase 758221 758868
NMB0728 phenylalanyl-tRNA synthetase, beta chain 758896 761256
NMB0729 integration host factor, alpha subunit 761333 761632
NMB0730 hypothetical protein 762257 762739
NMB0731 hypothetical protein 763002 763226
NMB0732 adenosylmethionine-8-amino-7-oxononanoate aminotransferase 763559
764857
NMB0733 dethiobiotin synthase 764857 765501
NMB0734 hypothetical protein 765519 765992
NMB0735 4-hydroxybenzoate octaprenyltransferase 766025 766912
NMB0736 PTS system, nitrogen regulatory IIA protein 767100 767546
NMB0737 HPr kinase/phosphatase, putative 767551 768510
NMB0738 conserved hypothetical protein 768494 769345
NMB0739 conserved hypothetical protein 769429 770943
NMB0740 DNA repair protein RecN 771255 772925
NMB0741 conserved hypothetical protein 775384 773948
NMB0742 conserved hypothetical protein 775684 776040
NMB0743 ubiquinone/menaquinone biosynthesis methyltransferase UbiE 776097
776831
NMB0744 hypothetical protein 777054 777530
NMB0745 2-amino-4-hydroxy-6-hydroxymethylidihydropteridine-
pyrophosphokinase 778153 777662
NMB0746 conserved hypothetical protein 778537 778166
NMB0747 conserved hypothetical protein 779157 778594
NMB0748 host factor-I 779535 779245
NMB0749 penicillin-binding protein 4 780602 779667
NMB0750 bacterioferritin comigratory protein 780923 781360
NMB0751 integrase/recombinase XerD 781415 782287
NMB0752 bacterioferritin-associated ferredoxin, putative 782462 782659
NMB0753 conserved hypothetical protein 782828 783058
NMB0754 hypothetical protein 783066 783173
NMB0755 hypothetical protein 783194 783334
NMB0756 dTDP-L-rhamnose synthase, putative 784398 783481
NMB0757 phosphoribosylaminoimidazole-succinocarboxamide synthase 784598
785458
NMB0758 polyribonucleotide nucleotidyltransferase 785695 787815
NMB0759 conserved hypothetical protein 788619 787894
NMB0760 diaminopimelate epimerase 789006 789854
NMB0761 hypothetical protein 789940 790164
NMB0762 hypothetical protein 790198 790653
NMB0763 cysteine synthase 790653 791582
NMB0764 conserved hypothetical protein 792048 792950
NMB0765 signal peptidase I 794128 793112
NMB0766 GTP-binding protein LepA 796064 794274
NMB0767 5-methylthioadenosine nucleosidase/S-adenosylhomocysteine
nucleosidase 796909 796211
NMB0768 twitching motility protein Pilt 797095 798204
NMB0769 DNA polymerase III, delta prime subunit, putative 798241 799215
NMB0770 type IV pilus assembly protein PilZ, putative 799222 799569
NMB0771 conserved hypothetical protein 799577 800353
NMB0772 conserved hypothetical protein 800382 800594
NMB0773 conserved hypothetical protein 800698 801006
NMB0774 uracil phosphoribosyltransferase 801115 801738
NMB0775 hypothetical protein 801764 802081
NMB0776 conserved hypothetical protein 802335 802751
NMB0777 uroporphyrinogen-III synthase HemD, putative 802796 803533
NMB0778 uroporphyrin-III C-methyltransferase HemX, putative 803611 804882
NMB0779 hypothetical protein 804882 806102
NMB0780 hypothetical protein 806138 806575
NMB0781 uroporphyrinogen decarboxylase 806732 807793
NMB0782 DNA repair protein RadA 807982 809358
NMB0783 conserved hypothetical protein 810116 809640
NMB0784 phage shock protein E precursor, putative 810717 810361

NMB0785 exodeoxyribonuclease V 135 KD polypeptide 814370 810759
NMB0786 conserved hypothetical protein 815358 814453
NMB0787 amino acid ABC transporter, periplasmic amino acid-binding protein
815643 816467
NMB0788 amino acid ABC transporter, permease protein 816514 817173
NMB0789 amino acid ABC transporter, ATP-binding protein 817186 817938
NMB0790 phosphoglucomutase 819343 817964
NMB0791 peptidyl-prolyl cis-trans isomerase 820019 819513
NMB0792 transporter, NadC family 821553 820141
NMB0793 hypothetical protein 821759 821553
NMB0794 hypothetical protein 822146 821787
NMB0795 peptidyl-tRNA hydrolase 822988 822413
NMB0796 conserved hypothetical protein 823319 823044
NMB0797 conserved hypothetical protein 823749 823315
NMB0798 cell division protein FtsH 825932 823968
NMB0799 cell division protein FtsJ 826616 825999
NMB0800 conserved hypothetical protein 826726 827007
NMB0801 delta-aminolevulinic acid dehydratase 827193 828191
NMB0802 cystathionine gamma-synthase 829414 828260
NMB0803 conserved hypothetical protein 829606 830376
NMB0804 NAD(P)H nitroreductase, putative 830489 831151
NMB0805 transposase, IS30 family 831295 832257
NMB0806 conserved hypothetical protein 833050 832295
NMB0807 conserved hypothetical protein 833965 833078
NMB0808 hypothetical protein 834551 833988
NMB0809 conserved hypothetical protein 835399 834605
NMB0810 transcriptional regulator, TetR family 836104 835457
NMB0811 UDP-N-acetylpyruvoylglucosamine reductase 837156 836119
NMB0812 conserved hypothetical protein 838579 837203
NMB0813 hypothetical protein 838634 838819
NMB0814 histidyl-tRNA synthetase 838914 840062
NMB0815 adenylosuccinate synthetase 840163 841464
NMB0816 hypothetical protein 841592 841903
NMB0817 hypothetical protein 841932 842312
NMB0818 hypothetical protein 842329 842736
NMB0819 hypothetical protein 842856 843245
NMB0820 hypothetical protein 843456 843845
NMB0821 hypothetical protein 843962 844519
NMB0822 heat shock protein HtpX 845866 844826
NMB0823 adenylate kinase 845878 846522
NMB0824 orotidine 5'-phosphate decarboxylase 847051 847788
NMB0825 ADP-heptose synthase, putative 847846 848814
NMB0826 C-5 cytosine-specific DNA methylase 848854 850086
NMB0827 type II restriction enzyme-related protein FRAMESHIFT 850091
851119
NMB0828 ADP-L-glycero-D-mannoheptose-6-epimerase 851251 852252
NMB0829 type I restriction enzyme EcoR124II M protein 852329 853870
NMB0830 conserved hypothetical protein 853870 854877
NMB0831 type I restriction enzyme S protein, degenerate 855046 856216
NMB0832 anticodon nuclease 856277 857416
NMB0833 type I restriction enzyme-related protein 857416 857799
NMB0834 transposase, IS30 family 858756 857794
NMB0835 type I restriction enzyme EcoR124II R protein, putative 858832
861594
NMB0836 ATP-dependent Clp protease, ATP-binding subunit ClpA 863945 861639
NMB0837 conserved hypothetical protein 864249 863950
NMB0838 cold-shock domain family protein 864492 864692
NMB0839 pmbA protein 866323 864995
NMB0840 conserved hypothetical protein 866446 866979
NMB0841 hypothetical protein 867029 867742
NMB0842 single-stranded-DNA-specific exonuclease RecJ 867814 869511
NMB0843 polyA polymerase 869811 871169
NMB0844 hypothetical protein 871345 871665
NMB0845 PhoH-related protein 872732 871782

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NMB0846 LPS biosynthesis protein-related protein 873905 872874
NMB0847 hypothetical protein 874235 874065
NMB0848 hypothetical protein 874369 875070
NMB0849 deoxycytidine triphosphate deaminase, putative 875703 875140
NMB0850 hypothetical protein 876185 875772
NMB0851 recombination associated protein RdcC 877146 876250
NMB0852 essential GTPase 878634 877180
NMB0853 conserved hypothetical protein 879413 878787
NMB0854 histidyl-tRNA synthetase 880709 879417
NMB0855 bacteriocin resistance protein, putative 881459 880806
NMB0856 hypothetical protein 882208 881744
NMB0857 hypothetical protein 882441 882268
NMB0858 hypothetical protein 882645 882448
NMB0859 hypothetical protein 883025 882651
NMB0860 hypothetical protein 883340 883086
NMB0861 hypothetical protein 883975 883433
NMB0862 hypothetical protein 884091 883975
NMB0863 hypothetical protein 884410 884141
NMB0864 hypothetical protein 884966 884679
NMB0865 hypothetical protein 885445 884975
NMB0866 hypothetical protein 886357 885491
NMB0867 YabO/YceC/SfhB family protein 886521 887441
NMB0868 conserved hypothetical protein 888163 887525
NMB0869 hypothetical protein 889009 888221
NMB0870 3-methyl-2-oxobutanoate hydroxymethyltransferase 889502 890290
NMB0871 pantoate--beta-alanine ligase 890416 891249
NMB0872 conserved hypothetical protein 891416 893257
NMB0873 outer membrane lipoprotein LolB, putative 893400 893978
NMB0874 conserved hypothetical protein 893991 894833
NMB0875 ribose-phosphate pyrophosphokinase 895258 896238
NMB0876 50S ribosomal protein L25 896308 896877
NMB0877 penicillin-binding protein 898174 897008
NMB0878 threonine dehydratase 898322 899845
NMB0879 sulfate ABC transporter, ATP-binding protein 900978 899908
NMB0880 sulfate ABC transporter, permease protein 901835 900978
NMB0881 sulfate ABC transporter, permease protein 902923 902090
NMB0882 hypothetical protein 903214 903543
NMB0883 conserved hypothetical protein 903878 904384
NMB0884 superoxide dismutase 905491 904907
NMB0885 replicative DNA helicase 905655 907058
NMB0886 fimbrial protein FimT 907370 908035
NMB0887 type IV pilus assembly protein PilV, putative 908056 908667
NMB0888 hypothetical protein 908667 909605
NMB0889 hypothetical protein 909587 910177
NMB0890 type IV pilin-related protein 910170 910655
NMB0891 hypothetical protein 911708 911944
NMB0892 AzlC-related protein 912795 912376
NMB0893 deoxyuridine 5'-triphosphate nucleotidohydrolase 912995 913444
NMB0894 aminotransferase, class I 913525 914709
NMB0895 conserved hypothetical protein 914975 915751
NMB0896 integrase, FRAMESHIFT 916283 917352
NMB0897 hypothetical protein 917468 917845
NMB0898 hypothetical protein 917894 918079
NMB0899 hypothetical protein 918396 918749
NMB0900 hypothetical protein 919621 920535
NMB0901 D-lactate dehydrogenase-related protein 920880 920599
NMB0902 hypothetical protein 921133 920945
NMB0903 hypothetical protein 921429 921139
NMB0904 hypothetical protein 921686 921429
NMB0905 hypothetical protein 921936 921724
NMB0906 hypothetical protein 922860 922009
NMB0907 hypothetical protein 923244 922888
NMB0908 hypothetical protein 923512 923315
NMB0909 hypothetical protein 924280 923759

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NMB0910 transcriptional regulator 925000 924287
NMB0911 transposase, IS30 family 926382 925420
NMB0912 hypothetical protein 926526 927149
NMB0913 pemK protein 927552 927208
NMB0914 pemI protein 927790 927557
NMB0915 hypothetical protein 928640 928152
NMB0916 hypothetical protein 928799 928662
NMB0917 death-on-curing protein 929446 929081
NMB0918 hypothetical protein 929574 929446
NMB0919 IS1106 transposase, putative 930929 929973
NMB0920 isocitrate dehydrogenase 934317 932095
NMB0921 hypothetical protein 934522 934325
NMB0922 alpha-2,3-sialyltransferase 934750 935862
NMB0923 cytochrome c 936488 936033
NMB0924 oxidoreductase, short-chain dehydrogenase/reductase family 936607
937425
NMB0925 acyl CoA thioester hydrolase family protein 937925 937482
NMB0926 opacity protein 940336 939513
NMB0927 proline iminopeptidase 941840 942769
NMB0928 hypothetical protein 944025 942832
NMB0929 dihydrodipicolinate synthase 944909 944037
NMB0930 xanthine/uracil permease family protein 945369 946757
NMB0931 RNA methyltransferase, TrmH family 947574 946825
NMB0932 conserved hypothetical protein 948129 947644
NMB0933 cytidine and deoxycytidylate deaminase family protein 948580
948137
NMB0934 DNA transformation protein tfoX-related protein 948853 948625
NMB0935 tRNA delta(2)-isopentenylpyrophosphate transferase 949798 948860
NMB0936 hypothetical protein 951481 950180
NMB0937 elongation factor P (EF-P) 951788 952345
NMB0938 hypothetical protein 953235 952402
NMB0939 conserved hypothetical protein 953933 953355
NMB0940 homoserine O-acetyltransferase 955069 953933
NMB0941 50S ribosomal protein L36 955756 955634
NMB0942 50S ribosomal protein L31, putative 956031 955759
NMB0943 5,10-methylenetetrahydrofolate reductase 956231 957106
NMB0944 5-methyltetrahydropteroyltriglutamate-homocysteine
methyltransferase 957247 959520
NMB0945 hypothetical protein 959535 959696
NMB0946 peroxiredoxin 2 family protein/glutaredoxin 959802 960536
NMB0947 lipoamide dehydrogenase, putative 960788 962188
NMB0948 succinate dehydrogenase, cytochrome b556 subunit 962470 962844
NMB0949 succinate dehydrogenase, hydrophobic membrane anchor protein
962841 963179
NMB0950 succinate dehydrogenase, flavoprotein subunit 963185 964945
NMB0951 succinate dehydrogenase, iron-sulfur protein 965068 965772
NMB0952 conserved hypothetical protein 965779 966024
NMB0953 hypothetical protein 966024 966104
NMB0954 citrate synthase 966139 967419
NMB0955 2-oxoglutarate dehydrogenase, E1 component 967627 970452
NMB0956 2-oxoglutarate dehydrogenase, E2 component, dihydrolipoamide
succinyltransferase 970555 971733
NMB0957 2-oxoglutarate dehydrogenase, E3 component, lipoamide
dehydrogenase 972101 973531
NMB0958 hypothetical protein 973659 973943
NMB0959 succinyl-CoA synthetase, beta subunit 974045 975208
NMB0960 succinyl-CoA synthetase, alpha subunit 975222 976109
NMB0961 funZ protein 978267 976675
NMB0962 excinuclease ABC, subunit A 981150 978304
NMB0963 phosphatidylserine decarboxylase precursor-related protein 981305
982099
NMB0964 TonB-dependent receptor 985503 983230
NMB0965 hypothetical protein 985832 985564

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NMB0966 para-aminobenzoate synthase glutamine amidotransferase component
II 985925 986512

NMB0967 anthranilate phosphoribosyltransferase 986579 987634

NMB0968 hypothetical protein 987644 987729

NMB0969 hypothetical protein 988030 987792

NMB0970 conserved hypothetical protein, FRAMESHIFT 988106 989527

NMB0971 hypothetical protein 989493 989780

NMB0972 hypothetical protein 989788 989982

NMB0973 hypothetical protein 989993 990274

NMB0974 hypothetical protein 990284 990559

NMB0975 hypothetical protein 990690 991004

NMB0976 TspB-related protein 990991 991383

NMB0977 modulator of drug activity B, putative 991676 992146

NMB0978 NAD(P) transhydrogenase, beta subunit 993742 992360

NMB0979 hypothetical protein 994205 993825

NMB0980 NAD(P) transhydrogenase, alpha subunit 995750 994212

NMB0981 phosphoserine phosphatase 996040 996870

NMB0982 chloride channel protein-related protein 997018 998157

NMB0983 phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP
cyclohydrolase 998324 999901

NMB0984 transposase, putative, degenerate 1000517 1001457

NMB0985 El6-related protein 1001522 1002016

NMB0986 hypothetical protein 1001997 1002425

NMB0987 N-acetylmuramoyl-L-alanine amidase, putative 1002736 1003278

NMB0988 hypothetical protein 1003278 1003478

NMB0989 hypothetical protein 1003484 1003645

NMB0990 hypothetical protein 1003859 1004260

NMB0991 IS1106 transposase 1005417 1004308

NMB0992 adhesin 1007326 1005554

NMB0993 rubredoxin 1009428 1009261

NMB0994 acyl-CoA dehydrogenase family protein 1011202 1010114

NMB0995 macrophage infectivity potentiator-related protein 1012020 1011340

NMB0996 hypothetical protein 1012411 1012043

NMB0997 D-lactate dehydrogenase 1014397 1012709

NMB0998 oxidoreductase, putative 1014921 1018751

NMB0999 NifR3/SMM1 family protein 1018935 1019933

NMB1000 IS1106 transposase, putative FRAMESHIFT 1020537 1021551

NMB1001 integrase protein, degenerate 1023183 1022614

NMB1002 hypothetical protein 1024370 1023498

NMB1003 hypothetical protein 1024711 1024418

NMB1004 hypothetical protein 1024962 1024720

NMB1005 hypothetical protein 1025179 1024958

NMB1006 hypothetical protein 1025360 1025184

NMB1007 transcriptional regulator 1025451 1025819

NMB1008 hypothetical protein 1025824 1026444

NMB1009 conserved hypothetical protein 1026440 1026631

NMB1010 hypothetical protein 1026658 1027218

NMB1011 hypothetical protein 1027252 1028196

NMB1012 hypothetical protein 1028284 1028784

NMB1013 hypothetical protein 1028801 1028971

NMB1014 conserved hypothetical protein 1029045 1029635

NMB1015 IS150 transposase, putative FRAMESHIFT 1029653 1030443

NMB1016 conserved hypothetical protein 1031794 1031192

NMB1017 sulfate ABC transporter, periplasmic sulfate-binding protein
1033574 1032522

NMB1018 conserved hypothetical protein 1034162 1033683

NMB1019 phosphoribosylaminoimidazole carboxylase, ATPase subunit 1035345
1034212

NMB1020 hypothetical protein 1035887 1035345

NMB1021 anthranilate synthase component I 1037359 1035887

NMB1022 transposase, IS30 family 1038444 1037482

NMB1023 conserved hypothetical protein 1039543 1038587

NMB1024 conserved hypothetical protein 1040502 1039639

NMB1025 conserved hypothetical protein 1040896 1040537

NMB1026 conserved hypothetical protein 1040971 1041447
NMB1027 dnaJ protein, truncation 1041473 1042192
NMB1028 conserved hypothetical protein 1042197 1043069
NMB1029 aspartate ammonia-lyase 1044541 1043147
NMB1030 conserved hypothetical protein 1045565 1045005
NMB1031 3-isopropylmalate dehydrogenase 1046798 1045731
NMB1032 type II restriction enzyme NlaIV 1047563 1046835
NMB1033 modification methylase NlaIV 1048850 1047582
NMB1034 3-isopropylmalate dehydratase, small subunit 1049666 1049028
NMB1035 hypothetical protein 1049982 1049731
NMB1036 3-isopropylmalate dehydratase, large subunit 1051488 1050082
NMB1037 glutamate--cysteine ligase 1051748 1053094
NMB1038 DNA repair protein RadC 1053220 1053894
NMB1039 conserved hypothetical protein 1053970 1054692
NMB1040 hypothetical protein 1054848 1056125
NMB1041 GTP-binding protein 1056133 1057308
NMB1042 cation transport ATPase, E1-E2 family 1057308 1059776
NMB1043 hypothetical protein 1059940 1060142
NMB1044 ferredoxin--NADP reductase 1061316 1060543
NMB1045 hypothetical protein 1062298 1061507
NMB1046 threonine synthase 1063753 1062347
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NMB1048 hypothetical protein 1065918 1064452
NMB1049 transcriptional regulator, putative 1066174 1067085
NMB1050 transposase, IS30 family 1068512 1067550
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NMB1052 dedA protein 1071207 1070566
NMB1053 class 5 outer membrane protein 1072189 1071374
NMB1054 IS1106 transposase, degenerate 1073920 1072988
NMB1055 serine hydroxymethyltransferase 1075474 1074227
NMB1056 hypothetical protein 1075753 1075544
NMB1057 gamma-glutamyltranspeptidase 1077776 1075959
NMB1058 conserved hypothetical protein FRAMESHIFT 1078161 1077902
NMB1059 conserved hypothetical protein 1078505 1078720
NMB1060 fructose-1,6-bisphosphatase 1079840 1078869
NMB1061 conserved hypothetical protein 1080931 1080089
NMB1062 conserved hypothetical protein 1081610 1081011
NMB1063 dihydroneopterin aldolase 1081666 1082019
NMB1064 conserved hypothetical protein 1082056 1082589
NMB1065 crcB protein 1083465 1083109
NMB1066 hypothetical protein 1084174 1083497
NMB1067 cell division protein FtsK 1084339 1087380
NMB1068 gamma-glutamyl phosphate reductase 1088870 1087611
NMB1069 glutamate 5-kinase 1089992 1088886
NMB1070 2-isopropylmalate synthase 1090477 1092027
NMB1071 conserved hypothetical protein 1092125 1092784
NMB1072 prolipoprotein diacylglycerol transferase 1093721 1092873
NMB1073 conserved hypothetical protein 1094922 1093795
NMB1074 acetylglutamate kinase 1095092 1095985
NMB1075 conserved hypothetical protein 1098302 1097637
NMB1076 DnaA-related protein 1098967 1098302
NMB1077 ABC transporter, ATP-binding protein, truncation 1099623 1099075
NMB1078 transcriptional regulator, UmuD/LexA family 1100312 1099875
NMB1079 hypothetical protein 1100580 1100425
NMB1080 ner protein FRAMESHIFT 1100802 1101061
NMB1081 bacteriophage transposase 1101126 1103108
NMB1082 hypothetical protein 1103120 1103317
NMB1083 bacteriophage DNA transposition protein B, putative 1103481
1104650
NMB1084 hypothetical protein 1104655 1105173
NMB1085 N-acetylmuramoyl-L-alanine amidase, putative 1105319 1105861
NMB1086 hypothetical protein 1106234 1106467
NMB1087 hypothetical protein 1106758 1107060
NMB1088 conserved hypothetical protein 1107278 1107111

NMB1089 hypothetical protein 1107506 1107841
NMB1090 hypothetical protein 1107856 1108119
NMB1091 hypothetical protein 1108119 1108313
NMB1092 hypothetical protein 1108319 1108822
NMB1093 hypothetical protein 1109412 1108825
NMB1094 hypothetical protein 1109497 1111044
NMB1095 conserved hypothetical protein 1111047 1112612
NMB1096 conserved hypothetical protein 1112602 1113894
NMB1097 cryptic Mu-phage G protein, putative 1114007 1114419
NMB1098 I protein, putative 1114653 1115711
NMB1099 transposase, IS30 family 1116767 1115805
NMB1100 hypothetical protein 1116795 1117274
NMB1101 conserved hypothetical protein 1117277 1117696
NMB1102 hypothetical protein 1117746 1118336
NMB1103 hypothetical protein 1118336 1118530
NMB1104 phage sheath protein 1118536 1119942
NMB1105 hypothetical protein 1120010 1120384
NMB1106 hypothetical protein 1120391 1120753
NMB1107 hypothetical protein 1121610 1121011
NMB1108 hypothetical protein 1121780 1123933
NMB1109 phage virion protein, putative 1123936 1125264
NMB1110 tail protein, 43 kDa 1125257 1126399
NMB1111 baseplate assembly protein V, putative 1126399 1127064
NMB1112 conserved hypothetical protein 1127168 1127512
NMB1113 conserved hypothetical protein FRAMESHIFT 1127529 1128580
NMB1114 conserved hypothetical protein 1128580 1129137
NMB1115 tail fibre protein, putative 1129151 1131121
NMB1116 hypothetical protein 1131560 1132084
NMB1117 hypothetical protein 1132350 1132204
NMB1118 conserved hypothetical protein 1132762 1132478
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NMB1120 hypothetical protein 1133426 1133719
NMB1121 conserved hypothetical protein 1133719 1133925
NMB1122 ABC transporter, ATP-binding protein FRAMESHIFT 1135181 1134041
NMB1198 conserved hypothetical protein 1199352 1198465
NMB1161 hypothetical protein 1167620 1167426
NMB1162 hypothetical protein 1168307 1167663
NMB1163 hypothetical protein 1168675 1168307
NMB1164 hypothetical protein 1169353 1168685
NMB1165 oxidoreductase, short chain dehydrogenase/reductase family 1170237
1169521
NMB1128 conserved hypothetical protein 1139597 1138287
NMB1167 hypothetical protein 1171869 1171666
NMB1168 phytoene synthase, putative 1172903 1172034
NMB1131 chaperone protein HscA 1142897 1141038
NMB1132 hypothetical protein 1143630 1142977
NMB1171 conserved hypothetical protein / ankyrin-related protein 1176464
1175706
NMB1172 ferredoxin, 2Fe-2S type 1176860 1176522
NMB1173 hypothetical protein 1177278 1177138
NMB1136 hypothetical protein 1146017 1145337
NMB1175 conserved hypothetical protein 1178247 1178053
NMB1176 conserved hypothetical protein 1178719 1178321
NMB1139 acetyl-CoA carboxylase, carboxyl transferase alpha subunit 1147851
1146895
NMB1140 mesJ protein FRAMESHIFT 1149229 1147948
NMB1179 RNA methyltransferase, TrmH family 1182124 1181516
NMB1180 hypothetical protein 1182411 1182178
NMB1181 hypothetical protein 1182945 1182583
NMB1182 hypothetical protein 1183262 1182960
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diaminopimelate ligase 1152664 1151291
NMB1146 biotin synthetase 1153923 1152874
NMB1185 hypothetical protein 1186675 1186043

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NMB1148 hypothetical protein 1154845 1154693
 NMB1187 hypothetical protein 1187052 1186912
 NMB1150 dihydroxy-acid dehydratase 1157144 1155288
 NMB1189 sulfite reductase hemoprotein, beta-component 1191122 1189356
 NMB1190 sulfite reductase (NADPH) flavoprotein, alpha component 1192963
 1191152
 NMB1153 sulfate adenylyltransferase, subunit 1 1162210 1160927, plasmid
 protein [REDACTED]
 NMB1192 sulfate adenylyltransferase, subunit 2 1195208 1194288
 NMB1155 phosphoadenosine phosphosulfate reductase 1163950 1163213
 NMB1194 siroheme synthase 1197448 1196000
 NMB1195 hypothetical protein 1197732 1197577
 NMB1158 nickel-dependent hydrogenase, b-type cytochrome subunit 1166365
 1165712
 NMB1197 conserved hypothetical protein 1199352 1198465
 NMB1199 GTP-binding protein TypA 1201433 1199625
 NMB1200 ribonuclease II family protein 1202272 1204644
 NMB1201 IMP dehydrogenase 1206449 1204989
 NMB1202 hypothetical protein 1207237 1206779
 NMB1203 protein-PII uridylyltransferase 1209886 1207331
 NMB1204 transcriptional regulator 1210255 1209938
 NMB1205 hypothetical protein 1210426 1210283
 NMB1206 bacterioferritin B 1211053 1210583
 NMB1207 bacterioferritin A 1211545 1211084
 NMB1208 hypothetical protein 1211610 1211810
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 NMB1211 hypothetical protein 1212984 1212745
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 NMB1213 hypothetical protein 1213678 1213319
 NMB1214 hemagglutinin/hemolysin-related protein 1220496 1213678
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 NMB1216 lipoleic acid synthetase 1221989 1221009
 NMB1217 lipoleate-protein ligase B 1222554 1221985
 NMB1218 conserved hypothetical protein 1222882 1222610
 NMB1219 transporter, putative 1223067 1224134
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 NMB1223 site-specific DNA methylase, degenerate 1226520 1229028
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 NMB1225 hypothetical protein 1230112 1229600
 NMB1226 ABC transporter, ATP-binding protein 1232500 1230581
 NMB1227 conserved hypothetical protein 1232972 1232580
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 NMB1230 DNA-binding protein HU-beta 1235207 1234941
 NMB1231 ATP-dependent protease La 1237851 1235392
 NMB1232 conserved hypothetical protein 1238285 1239202
 NMB1233 exodeoxyribonuclease V, alpha subunit 1240978 1239236
 NMB1234 ABC transporter, ATP-binding protein 1241741 1241049
 NMB1235 conserved hypothetical protein 1242981 1241737
 NMB1236 hypothetical protein 1243186 1243461
 NMB1237 recombination protein RecR 1244140 1243523
 NMB1238 peptidyl-prolyl cis-trans isomerase-related protein 1245742
 1244207
 NMB1239 conserved hypothetical protein 1246176 1245805
 NMB1240 ABC transporter, ATP-binding protein 1246326 1247951
 NMB1241 tRNA nucleotidyltransferase 1248026 1249276
 NMB1242 hypothetical protein 1249502 1249807
 NMB1243 Holliday junction DNA helicase RuvB 1249892 1250920
 NMB1244 ribulose-phosphate 3-epimerase 1251674 1250949
 NMB1245 hypothetical protein 1252367 1252035
 NMB1246 conserved hypothetical protein 1253294 1252434

NMB1247 riboflavin synthase, alpha subunit 1254006 1253305
NMB1248 molybdopterin-guanine dinucleotide biosynthesis protein A
FRAMESHIFT 1254659 1254085
NMB1249 nitrate/nitrite sensory protein NarX, putative 1254901 1256670
NMB1250 transcriptional regulator, LuxR family 1256670 1257323
NMB1251 transposase, IS30 family 1258731 1257769
NMB1252 phosphoribosylformylglycinamide cyclo-ligase 1259914 1258883
NMB1253 hypothetical protein 1260672 1261346
NMB1254 GTP cyclohydrolase II 1261342 1261932
NMB1255 glycosyl transferase, degenerate 1262256 1263263
NMB1256 GTP cyclohydrolase II/3,4-dihydroxy-2-butanone-4-phosphate
synthase 1263728 1264816
NMB1257 site-specific DNA methylase, degenerate 1265357 1265130
NMB1258 conserved hypothetical protein 1267046 1265739
NMB1259 transposase, IS30 family 1267584 1268546
NMB1260 type III restriction-modification system EcoPI enzyme, subunit res
1271565 1268629
NMB1261 type III restriction-modification system EcoPI enzyme, subunit mod
POINT MUTATION FRAMESHIFT 1273661 1271581
NMB1262 peptidyl-prolyl cis-trans isomerase 1274334 1273780
NMB1263 CobW-related protein 1275316 1274402
NMB1264 conserved hypothetical protein 1275771 1275502
NMB1265 conserved hypothetical protein 1276061 1275771
NMB1266 zinc uptake regulation protein, putative 1276582 1276109
NMB1267 low molecular weight protein tyrosine-phosphatase 1277108 1276656
NMB1268 conserved hypothetical protein 1278348 1277236
NMB1269 hypothetical protein 1279559 1278465
NMB1270 conserved hypothetical protein 1281272 1279644
NMB1271 mercury transport periplasmic protein, putative 1281584 1281375
NMB1272 hypothetical protein 1281765 1281625
NMB1273 alginate O-acetylation protein AlgI, putative 1282215 1283648
NMB1274 hypothetical protein 1283662 1284642
NMB1275 hypothetical protein 1284642 1286083
NMB1276 long-chain-fatty-acid--CoA ligase 1286122 1287672
NMB1277 transporter, BCCT family 1289792 1287768
NMB1278 site-specific recombinase 1290081 1292084
NMB1279 membrane-bound lytic murein transglycosylase B, putative 1293319
1292213
NMB1280 very long chain acyl-CoA dehydrogenase-related protein 1294948
1293524
NMB1281 transcription-repair coupling factor 1295133 1299269
NMB1282 aspartate 1-decarboxylase 1299421 1299801
NMB1283 2-dehydro-3-deoxyphosphooctonate aldolase 1299826 1300665
NMB1284 hypothetical protein 1300683 1301120
NMB1285 enolase 1301171 1302454
NMB1286 conserved hypothetical protein 1302471 1302746
NMB1287 ferredoxin, putative 1303080 1302793
NMB1288 ribonucleoside-diphosphate reductase, beta subunit 1304479 1303328
NMB1289 type II restriction enzyme, putative 1305706 1304522
NMB1290 C-5 cytosine-specific DNA-methylase 1306712 1305702
NMB1291 ribonucleoside-diphosphate reductase, alpha subunit 1309049
1306773
NMB1292 hypothetical protein 1309394 1309209
NMB1293 hypothetical protein 1309563 1309886
NMB1294 1-acyl-sn-glycerol-3-phosphate acyltransferase 1310967 1310203
NMB1295 formamidopyrimidine-DNA glycosylase 1311882 1311058
NMB1296 hypothetical protein 1312599 1311937
NMB1297 membrane-bound lytic murein transglycosylase D 1312778 1314751
NMB1298 ribosomal small subunit pseudouridine synthase A 1314822 1315511
NMB1299 sodium- and chloride-dependent transporter, degenerate 1316091
1317454
NMB1300 cytidylate kinase 1317701 1318354
NMB1301 30S ribosomal protein S1 1318513 1320195
NMB1302 integration host factor, beta subunit 1320209 1320520

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NMB1303 transcriptional regulator, MerR family 1321281 1320877
 NMB1304 alcohol dehydrogenase, class III 1321402 1322535
 NMB1305 esterase, putative 1322547 1323371
 NMB1306 conserved hypothetical protein 1323765 1324913
 NMB1307 nucleoside diphosphate kinase 1324975 1325397
 NMB1308 conserved hypothetical protein 1325543 1326634
 NMB1309 fimbrial biogenesis and twitching motility protein, putative
 1326640 1327398
 NMB1310 gcpE protein 1327417 1328679
 NMB1311 hypothetical protein 1328970 1328737
 NMB1312 ATP-dependent Clp protease, proteolytic subunit 1329655 1329128
 NMB1313 trigger factor 1331148 1329838
 NMB1314 cell division protein FtsK 1333791 1331356
 NMB1315 uracil permease 1334014 1335222
 NMB1316 hypothetical protein 1335289 1335726
 NMB1317 hypothetical protein 1335865 1336266
 NMB1318 CDP-diacylglycerol--serine O-phosphatidyltransferase 1336343
 1337086
 NMB1319 conserved hypothetical protein 1337090 1337860
 NMB1320 50S ribosomal protein L9 1338540 1338091
 NMB1321 30S ribosomal protein S18 1338787 1338560
 NMB1322 primosomal replication protein n, putative 1339096 1338797
 NMB1323 30S ribosomal protein S6 1339465 1339100
 NMB1324 thioredoxin reductase 1340571 1339624
 NMB1325 cation transport ATPase, E1-E2 family 1340710 1342869
 NMB1326 excinuclease ABC, subunit C 1342969 1344819
 NMB1327 conserved hypothetical protein 1345045 1346445
 NMB1328 conserved hypothetical protein 1346570 1347283
 NMB1329 hypothetical protein 1347649 1347840
 NMB1330 hypothetical protein 1348276 1347917
 NMB1331 excinuclease ABC, subunit B 1350416 1348392
 NMB1332 carboxy-terminal peptidase 1352229 1350748
 NMB1333 conserved hypothetical protein 1354146 1352359
 NMB1334 hypothetical protein 1354238 1354471
 NMB1335 creA protein 1354474 1355031
 NMB1336 conserved hypothetical protein 1355036 1355581
 NMB1337 conserved hypothetical protein 1355577 1356029
 NMB1338 isomerase, putative 1356698 1356045
 NMB1339 prolyl-tRNA synthetase 1358473 1356764
 NMB1340 hypothetical protein 1358924 1359151
 NMB1341 pyruvate dehydrogenase, E1 component 1359167 1361827
 NMB1342 pyruvate dehydrogenase, E2 component, dihydrolipoamide
 acetyltransferase FRAMESHIFT 1361979 1363583
 NMB1343 hypothetical protein 1363680 1364114
 NMB1344 pyruvate dehydrogenase, E3 component, lipoamide dehydrogenase
 1364135 1365916
 NMB1345 hypothetical protein 1367830 1366283
 NMB1346 TonB-dependent receptor, putative FRAMESHIFT 1369731 1367957
 NMB1347 extragenic suppressor protein SuhB 1370786 1370004
 NMB1348 RNA methylase, putative 1371030 1371842
 NMB1349 hypothetical protein 1371906 1372760
 NMB1350 hypothetical protein 1372967 1373305
 NMB1351 fmu and fmv protein, putative 1373656 1374909
 NMB1352 hypothetical protein 1375272 1375703
 NMB1353 aldehyde dehydrogenase family protein 1377097 1375757
 NMB1354 conserved hypothetical protein 1377755 1377105
 NMB1355 glutamyl-tRNA (Gln) amidotransferase subunit C, putative 1377906
 1378193
 NMB1356 Glu-tRNA(Gln) amidotransferase, subunit A 1378259 1379701
 NMB1357 conserved hypothetical protein 1379701 1380630
 NMB1358 Glu-tRNA(Gln) amidotransferase, subunit B 1380676 1382103
 NMB1359 CDP-6-deoxy-delta-3,4-glucoseen reductase, putative 1382318
 1383325
 NMB1360 pyridoxamine 5-phosphate oxidase 1384090 1383461

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NMB1361 conserved hypothetical protein 1384312 1385361
NMB1362 oxalate/formate antiporter, putative 1386974 1385436
NMB1363 exodeoxyribonuclease, large subunit 1388622 1387270
NMB1364 NH(3)-dependent NAD⁺ synthetase NadE, putative 1388819 1389637
NMB1365 conserved hypothetical protein 1390183 1389713
NMB1366 thioredoxin 1390481 1390810
NMB1367 conserved hypothetical protein 1391930 1390869
NMB1368 ATP-dependent RNA helicase, putative 1392141 1393526
NMB1369 hypothetical protein 1394572 1394021
NMB1370 hypothetical protein 1395217 1394860
NMB1371 acetylornithine aminotransferase 1395561 1396754
NMB1372 ATP-dependent Clp protease, ATP-binding subunit ClpX 1398104
1396863
NMB1373 ribosome-binding factor A 1398295 1398663
NMB1374 tRNA pseudouridine synthase B 1398699 1399619
NMB1375 modification methylase, putative FRAMESHIFT 1399839 1401945
NMB1376 conserved hypothetical protein POINT MUTATION 1401938 1404712
NMB1377 L-lactate dehydrogenase 1406036 1404867
NMB1378 conserved hypothetical protein 1406327 1406770
NMB1379 nifs protein 1406802 1408013
NMB1380 nifU protein 1408280 1408663
NMB1381 HesB/YadR/YfhF family protein 1408693 1409070
NMB1382 conserved hypothetical protein 1409254 1409036
NMB1383 chaperone protein HscB 1409336 1409833
NMB1384 DNA gyrase subunit A 1409934 1412681
NMB1385 IS1016 family transposase, degenerate 1412841 1413241
NMB1386 transposase, putative FRAMESHIFT 1413303 1413955
NMB1387 hypothetical protein 1414840 1414292
NMB1388 glucose-6-phosphate isomerase 1416500 1414857
NMB1389 RpiR/YebK/YfhH family protein 1417469 1416624
NMB1390 glucokinase 1418505 1417522
NMB1391 oxidoreductase, Sol/DevB family 1419181 1418489
NMB1392 glucose-6-phosphate 1-dehydrogenase 1420906 1419464
NMB1393 phosphogluconate dehydratase 1421474 1423306
NMB1394 4-hydroxy-2-oxoglutarate aldolase/2-dehydro-3-deoxyphosphogluconate
aldolase 1423490 1424125
NMB1395 alcohol dehydrogenase, zinc-containing 1425427 1424390
NMB1396 A/G-specific adenine glycosylase 1425581 1426627
NMB1397 hypothetical protein 1426793 1426972
NMB1398 Cu-Zn-superoxide dismutase 1427047 1427604
NMB1399 IS1106 transposase 1429146 1428175
NMB1400 ABC transporter family protein 1431631 1429406
NMB1401 IS1016C2 transposase 1432983 1432447
NMB1402 hypothetical protein 1433320 1433751
NMB1403 FrpA/C-related protein 1433795 1433983
NMB1404 hypothetical protein 1434021 1434746
NMB1405 FrpA/C-related protein 1434763 1435962
NMB1406 hypothetical protein 1436396 1436755
NMB1407 FrpA-related protein, degenerate 1436755 1437881
NMB1408 hypothetical protein 1437960 1438451
NMB1409 FrpA/C-related protein 1438582 1439007
NMB1410 hypothetical protein 1439247 1439783
NMB1411 IS1016C2 transposase 1440610 1439960
NMB1412 FrpC operon protein 1441216 1442022
NMB1413 IS1016 family transposase, putative FRAMESHIFT 1442715 1442132
NMB1414 FrpC operon protein 1442798 1443568
NMB1415 iron-regulated protein FrpC 1443588 1449074
NMB1416 aminopeptidase N 1452022 1449422
NMB1417 conserved hypothetical protein 1452947 1452156
NMB1418 HtrB/MsbB family protein 1454563 1453697
NMB1419 crossover junction endodeoxyribonuclease RuvC 1455150 1454617
NMB1420 factor-for-inversion stimulation protein Fis, putative 1455392
1455156
NMB1421 nifR3 protein 1456432 1455425

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NMB1422 ATP-dependent RNA helicase, putative 1456798 1458168
NMB1423 conserved hypothetical protein 1458746 1459870
NMB1424 hypothetical protein 1459903 1460928
NMB1425 lysyl-tRNA synthetase, heat inducible 1462560 1461052
NMB1426 hypothetical protein 1463968 1462718
NMB1427 hypothetical protein 1464208 1464032
NMB1428 aminopeptidase, putative 1464426 1466219
NMB1429 outer membrane protein PorA 1468209 1467034
NMB1430 transcription elongation factor GreA 1470964 1470491
NMB1431 hypothetical protein 1471298 1471050
NMB1432 3-phosphoshikimate 1-carboxyvinyltransferase 1471360 1472658
NMB1433 conserved hypothetical protein FRAMESHIFT 1473237 1472707
NMB1434 cardiolipin synthetase family protein 1474971 1473448
NMB1435 drug resistance translocase family protein 1476489 1475086
NMB1436 conserved hypothetical protein 1476774 1477550
NMB1437 conserved hypothetical protein 1477550 1478248
NMB1438 conserved hypothetical protein 1478248 1479699
NMB1439 phosphoribosylaminoimidazole carboxylase, catalytic subunit
1480370 1479888
NMB1440 hypothetical protein 1481131 1480421
NMB1441 O-methyltransferase, putative 1481799 1481134
NMB1442 mismatch repair protein MutL 1482139 1484112
NMB1443 DNA polymerase III, subunits gamma and tau 1484210 1486321
NMB1444 conserved hypothetical protein 1486404 1486736
NMB1445 recA protein 1489556 1488513
NMB1446 3-dehydroquinate dehydratase 1489810 1490571
NMB1447 ATP-dependent DNA helicase Rep 1490594 1492606
NMB1448 DNA-damage-inducible protein P 1493734 1492781
NMB1449 TonB-dependent receptor POINT MUTATION 1496967 1493881
NMB1450 ferredoxin--NADP reductase 1497241 1498017
NMB1451 DNA polymerase III, epsilon subunit 1499643 1498234
NMB1452 conserved hypothetical protein 1500459 1501595
NMB1453 hypothetical protein 1502335 1501847
NMB1454 ferredoxin, 4Fe-4S bacterial type 1503891 1502398
NMB1455 hypothetical protein 1504075 1503959
NMB1456 hypothetical protein 1504347 1504153
NMB1457 transketolase 1504419 1506395
NMB1458 fumarate hydratase, class II 1506547 1507932
NMB1459 conserved hypothetical protein 1508923 1508003
NMB1460 single-strand binding protein 1509972 1509451
NMB1461 drug resistance translocase family protein 1511361 1509979
NMB1462 transglycosylase, putative 1512092 1511472
NMB1463 IS1106 transposase, degenerate 1512998 1512596
NMB1464 conserved hypothetical protein 1513541 1513053
NMB1465 opacity protein FRAMESHIFT 1515309 1514483
NMB1466 conserved hypothetical protein 1515639 1516367
NMB1467 exopolyphosphatase 1516487 1517992
NMB1468 hypothetical protein 1518527 1518207
NMB1469 hypothetical protein 1518607 1518527
NMB1470 hypothetical protein 1519392 1518850
NMB1471 tryptophanyl-tRNA synthetase 1520471 1519464
NMB1472 clpB protein 1520732 1523308
NMB1473 aminotransferase, class I 1524612 1523401
NMB1474 4-oxalocrotonate tautomerase, putative 1524910 1524704
NMB1475 conserved hypothetical protein 1525255 1526058
NMB1476 glutamate dehydrogenase, NAD-specific 1527384 1526122
NMB1477 hypothetical protein 1527562 1527396
NMB1478 phosphoglycolate phosphatase FRAMESHIFT 1527786 1528489
NMB1479 regulatory protein RecX 1528560 1529018
NMB1480 hypothetical protein 1529095 1529253
NMB1481 hypothetical protein 1529262 1529393
NMB1482 acyl CoA thioester hydrolase family protein 1529409 1529888
NMB1483 lipoprotein NlpD, putative 1531499 1530255
NMB1484 stationary-phase survival protein SurE 1532501 1531758

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NMB1485 conserved hypothetical protein 1534074 1532521
NMB1486 hypothetical protein 1534263 1534126
NMB1487 fimbrial assembly protein 1535230 1534445
NMB1488 succinate-semialdehyde dehydrogenase (NADP+) 1536772 1535342
NMB1489 hypothetical protein 1537259 1537750
NMB1490 hypothetical protein 1538345 1537917
NMB1491 hypothetical protein 1538785 1538699
NMB1492 hypothetical protein 1538860 1538795
NMB1493 carbon starvation protein A 1538892 1540970
NMB1494 conserved hypothetical protein 1540963 1541154
NMB1495 hypothetical protein 1541371 1541562
NMB1496 conserved hypothetical protein 1541673 1542230
NMB1497 TonB-dependent receptor 1543234 1545996
NMB1498 aspartokinase, alpha and beta subunits 1549220 1548006
NMB1499 ribonuclease PH 1550148 1549423
NMB1500 conserved hypothetical protein 1550694 1550233
NMB1501 HsaA/MoeB/ThiF family protein 1550911 1551684
NMB1502 hypothetical protein 1551825 1552349
NMB1503 hypothetical protein 1552608 1552814
NMB1504 conserved hypothetical protein 1552706 1553557
NMB1505 nicotinate phosphoribosyltransferase 1553601 1554806
NMB1506 arginyl-tRNA synthetase 1554901 1556616
NMB1507 hypothetical protein 1556714 1557070
NMB1508 hypothetical protein 1557130 1558584
NMB1509 amino acid ABC transporter, permease protein 1560344 1559601
NMB1510 thermonuclease family protein 1561224 1560526
NMB1511 ribose 5-phosphate isomerase A 1561934 1561266
NMB1512 YgbB/YacN family protein 1562493 1562014
NMB1513 conserved hypothetical protein 1563214 1562528
NMB1514 DNA polymerase III, epsilon subunit 1563945 1563214
NMB1515 transporter, putative 1565411 1564104
NMB1516 fixS protein 1565589 1565404
NMB1517 hypothetical protein 1565885 1565589
NMB1518 acetate kinase 1566236 1567429
NMB1519 thiol:disulfide interchange protein DsbD 1569752 1567950
NMB1520 hypothetical protein 1570337 1569819
NMB1521 phytoene synthase-related protein 1571249 1570425
NMB1522 FKBP-type peptidyl-prolyl cis-trans isomerase SlyD 1571803 1571324
NMB1523 hypothetical protein 1572276 1572569
NMB1524 oxidoreductase, putative 1572682 1574046
NMB1525 VirG-related protein FRAMESHIFT 1576262 1574233
NMB1526 small major protein B 1577081 1576638
NMB1527 ADP-heptose--LPS heptosyltransferase II 1578146 1577139
NMB1528 methylated-DNA--protein-cysteine methyltransferase, putative
1579353 1578547
NMB1529 conserved hypothetical protein FRAMESHIFT 1579597 1580409
NMB1530 succinyl-diaminopimelate desuccinylase 1582228 1581086
NMB1531 conserved hypothetical protein 1582961 1582344
NMB1532 conserved hypothetical protein 1583504 1582998
NMB1533 H.8 outer membrane protein 1584150 1583602
NMB1534 hypothetical protein 1584287 1584150
NMB1535 hypothetical protein 1584404 1584874
NMB1536 preprotein translocase SecA subunit 1584984 1587731
NMB1537 DNA primase 1587879 1589648
NMB1538 RNA polymerase sigma factor RpoD 1589838 1591763
NMB1539 IS1106 transposase 1591913 1592917
NMB1540 lactoferrin-binding protein A 1597271 1594443
NMB1541 lactoferrin-binding protein B 1599481 1597271
NMB1542 hypothetical protein 1600504 1600722
NMB1543 conserved hypothetical protein 1600871 1602082
NMB1544 hypothetical protein 1602097 1602405
NMB1545 hypothetical protein 1602412 1602609
NMB1546 hypothetical protein 1602795 1603076
NMB1547 hypothetical protein 1603107 1603406

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NMB1548 tspB protein, putative 1603741 1605384
NMB1549 hypothetical protein 1606176 1606325
NMB1550 conserved hypothetical protein 1606332 1606613
NMB1551 conserved hypothetical protein 1606617 1607717
NMB1552 pilin gene inverting protein PivNM-1A 1608019 1608972
NMB1553 transposase, truncation 1612022 1611708
NMB1554 CTP synthase 1613884 1612253
NMB1555 long-chain-fatty-acid--CoA ligase 1615666 1613999
NMB1556 tRNA (5-methylaminomethyl-2-thiouridylate) -methyltransferase
1616840 1615740
NMB1557 conserved hypothetical protein 1617439 1616969
NMB1558 diacylglycerol kinase 1618115 1617735
NMB1559 glutathione synthetase 1619386 1618430
NMB1560 glutaminyl-tRNA synthetase 1621164 1619479
NMB1561 transcriptional regulator, DeoR family 1622049 1621279
NMB1562 conserved hypothetical protein 1622994 1622095
NMB1563 transcriptional regulator, GntR family 1623859 1623146
NMB1564 conserved hypothetical protein 1624850 1624431
NMB1565 hypothetical protein 1625639 1624971
NMB1566 phosphoribosylglycinamide formyltransferase 1626281 1625658
NMB1567 macrophage infectivity potentiator 1627206 1626391
NMB1568 DNA polymerase holoenzyme chi subunit, putative 1627905 1627468
NMB1569 aminopeptidase A/I, FRAMESHIFT 1629499 1627971
NMB1570 conserved hypothetical protein 1629544 1630656
NMB1571 conserved hypothetical protein 1630656 1631723
NMB1572 aconitate hydratase 2 1631936 1634518
NMB1573 ornithine carbamoyltransferase, catabolic 1634663 1635655
NMB1574 ketol-acid reductoisomerase 1636895 1635885
NMB1575 conserved hypothetical protein 1637268 1636978
NMB1576 acetolactate synthase III, small subunit 1637826 1637338
NMB1577 acetolactate synthase III, large subunit 1639564 1637840
NMB1578 conserved hypothetical protein 1640685 1641335
NMB1579 ATP phosphoribosyltransferase 1641417 1642067
NMB1580 hypothetical protein 1642174 1643070
NMB1581 histidinol dehydrogenase 1643070 1644356
NMB1582 histidinol-phosphate aminotransferase 1644405 1645499
NMB1583 imidazoleglycerol-phosphate dehydratase 1645499 1646413
NMB1584 3-hydroxyacid dehydrogenase 1646511 1647377
NMB1585 transcriptional regulator, MarR family 1647658 1648086
NMB1586 hypothetical protein 1648100 1648963
NMB1587 protease, putative 1650120 1649020
NMB1588 CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase
1651479 1650919
NMB1589 hypothetical protein 1652036 1651797
NMB1590 conserved hypothetical protein 1652675 1652343
NMB1591 transcriptional regulator MtrA 1652804 1653706
NMB1592 hypothetical protein 1653729 1654313
NMB1593 conserved hypothetical protein 1654445 1655305
NMB1594 spermidine/putrescine ABC transporter, periplasmic
spermidine/putrescine-binding protein 1656479 1655352
NMB1595 alanyl-tRNA synthetase 1656684 1659305
NMB1596 hypothetical protein 1659348 1659551
NMB1597 hypothetical protein 1659569 1659997
NMB1598 hypothetical protein 1660094 1660282
NMB1599 hypothetical protein 1660300 1660584
NMB1600 hypothetical protein 1660624 1660878
NMB1601 IS1106 transposase 1661075 1662079
NMB1602 transposase, putative 1663112 1661997
NMB1603 tellurite resistance protein, putative 1663289 1664230
NMB1604 phosphoglycerate mutase 1664989 1664309
NMB1605 topoisomerase IV subunit A 1665137 1667437
NMB1606 sensor histidine kinase 1667460 1669033
NMB1607 sigma-54 dependent response regulator 1669029 1669493
NMB1608 conserved hypothetical protein 1669600 1670349

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NMB1609 trans-sulfuration enzyme family protein 1672860 1671694
NMB1610 hypothetical protein 1673766 1673008
NMB1611 hypothetical protein 1673866 1674114
NMB1612 amino acid ABC transporter, periplasmic amino acid-binding protein
1674169 1674972
NMB1613 fumarate hydratase, class I 1675282 1676802
NMB1614 Trk system potassium uptake protein TrkA 1676903 1678312
NMB1615 hypothetical protein 1678758 1679018
NMB1616 phosphomethylpyrimidine kinase 1679755 1680558
NMB1617 tellurite resistance protein, putative 1681480 1680614
NMB1618 ribonuclease HI 1681594 1682028
NMB1619 conserved hypothetical protein 1682889 1683290
NMB1620 conserved hypothetical protein 1683333 1684514
NMB1621 glutathione peroxidase 1685113 1684583
NMB1622 nitric oxide reductase 1687547 1685295
NMB1623 major anaerobically induced outer membrane protein 1687918 1689087
NMB1624 conserved hypothetical protein 1689215 1689967
NMB1625 pilin gene inverting protein PivNM-1B 1691651 1690698
NMB1626 conserved hypothetical protein 1693053 1691953
NMB1627 conserved hypothetical protein 1693338 1693057
NMB1628 tspB protein, putative 1695347 1693797
NMB1629 Hypothetical protein 1695690 1695328
NMB1630 hypothetical protein 1696057 1695758
NMB1631 hypothetical protein 1696449 1696088
NMB1632 hypothetical protein 1696752 1696555
NMB1633 hypothetical protein 1697067 1696759
NMB1634 conserved hypothetical protein 1698296 1697091
NMB1635 hypothetical protein 1698662 1698444
NMB1636 opacity protein FRAMESHIFT 1700231 1701047
NMB1637 conserved hypothetical protein 1701808 1701254
NMB1638 YhbX/YhjW/YijP/YjdB family protein 1703518 1701887
NMB1639 hypothetical protein 1703921 1703595
NMB1640 phosphoserine aminotransferase 1705027 1703924
NMB1641 conserved hypothetical protein 1705374 1705820
NMB1642 N utilization substance protein A 1705851 1707350
NMB1643 translation initiation factor IF-2 1707365 1710250
NMB1644 hypothetical protein 1711755 1710418
NMB1645 hypothetical protein 1713169 1711832
NMB1646 hemolysin, putative 1713312 1713935
NMB1647 amino acid symporter, putative 1715420 1714005
NMB1648 conserved hypothetical protein 1715747 1716472
NMB1649 disulfide bond formation protein B 1717022 1716537
NMB1650 leucine-responsive regulatory protein 1718177 1717716
NMB1651 alanine racemase 1718502 1719557
NMB1652 conserved hypothetical protein 1720979 1719627
NMB1653 conserved hypothetical protein 1721266 1720997
NMB1654 conserved hypothetical protein 1722129 1721395
NMB1655 adenine specific methylase, putative 1723321 1722413
NMB1656 hypothetical protein 1723454 1724044
NMB1657 comE operon protein 1-related protein 1725327 1724713
NMB1658 DNA/pantothenate metabolism flavoprotein 1731065 1732246
NMB1659 guanosine-3',5'-bis(diphosphate) 3'-pyrophosphohydrolase 1734472
1732319
NMB1660 DNA-directed RNA polymerase, omega subunit 1734770 1734567
NMB1661 guanylate kinase 1735446 1734832
NMB1662 adenine phosphoribosyltransferase 1735607 1736170
NMB1663 conserved hypothetical protein 1737007 1736222
NMB1664 protease, putative 1737332 1738684
NMB1665 conserved hypothetical protein 1739253 1738870
NMB1666 hypothetical protein 1739498 1739253
NMB1667 hypothetical protein 1740061 1739858
NMB1668 hemoglobin receptor 1742596 1740224
NMB1669 iron-starvation protein PigA 1743420 1742794
NMB1670 PqiA family protein 1743706 1745214

NMB1671 pqiB protein 1745210 1746868
NMB1672 conserved hypothetical protein 1746871 1747386
NMB1673 DNA-3-methyladenine glycosylase I, putative 1747393 1747941
NMB1674 GDSL lipase family protein 1747934 1748572
NMB1675 hypothetical protein 1748797 1749102
NMB1676 glycine dehydrogenase (decarboxylating) 1749136 1751984
NMB1677 cytochrome c5 1753288 1752452
NMB1678 aromatic-amino-acid aminotransferase 1754906 1753716
NMB1679 tRNA (uracil-5-)-methyltransferase 1756015 1754930
NMB1680 chorismate synthase 1756162 1757259
NMB1681 hypothetical protein 1757354 1757776
NMB1682 topoisomerase IV subunit B 1759838 1757856
NMB1683 MutT/nudix family protein 1760429 1759908
NMB1684 seryl-tRNA synthetase 1760595 1761887
NMB1685 D-lactate dehydrogenase 1762966 1761971
NMB1686 peptide chain release factor 1 1764167 1763094
NMB1687 conserved hypothetical protein 1765042 1764275
NMB1688 L-asparaginase I 1766051 1765053
NMB1689 dedA protein, putative 1767007 1766327
NMB1690 phosphoglucomutase/phosphomannomutase family protein 1768532
1767201
NMB1691 dihydropteroate synthase 1769519 1768665
NMB1692 chorismate mutase-related protein 1770552 1769662
NMB1693 hypothetical protein 1770643 1772754
NMB1694 conserved hypothetical protein 1774305 1772824
NMB1695 hypothetical protein 1774424 1775401
NMB1696 acyl carrier protein 1775800 1775558
NMB1697 acyl carrier protein, putative 1776072 1775815
NMB1698 acyltransferase, putative 1776827 1776072
NMB1699 hypothetical protein 1777185 1776823
NMB1700 hypothetical protein 1777345 1777707
NMB1701 hypothetical protein 1777763 1778260
NMB1702 3-oxoacyl-(acyl-carrier-protein) reductase 1778291 1779016
NMB1703 3-oxoacyl-(acyl-carrier-protein) synthase II 1779013 1780260
NMB1704 beta-1,4-glucosyltransferase 1780467 1781222
NMB1705 alpha-1,2-N-acetylglucosamine transferase 1781226 1782287
NMB1706 hypothetical protein 1782329 1782496
NMB1707 sodium- and chloride-dependent transporter 1782677 1784011
NMB1708 NosX-related protein 1784846 1784189
NMB1709 thymidylate synthase 1785648 1784857
NMB1710 glutamate dehydrogenase, NADP-specific 1786032 1787363
NMB1711 transcriptional regulator, GntR family 1788280 1787504
NMB1712 L-lactate permease-related protein 1788711 1789007
NMB1713 transposase, IS30 family 1790361 1789399
NMB1714 multidrug efflux pump channel protein 1791874 1790474
NMB1715 multiple transferable resistance system protein MtrD 1795132
1791932
NMB1716 membrane fusion protein 1796382 1795147
NMB1717 transcriptional regulator MtrR 1796785 1797414
NMB1718 hypothetical protein 1797953 1797699
NMB1719 efflux pump component MtrF 1798240 1799805
NMB1720 exodeoxyribonuclease V 125 kD polypeptide 1803085 1799879
NMB1721 conserved hypothetical protein 1804596 1803190
NMB1722 cytochrome C555 FRAMESHIFT 1804923 1804801
NMB1723 cytochrome c oxidase, subunit III 1806129 1805035
NMB1724 cytochrome c oxidase, subunit II 1806939 1806331
NMB1725 cytochrome c oxidase, subunit I 1808411 1806969
NMB1726 conserved hypothetical protein 1808726 1810471
NMB1727 conserved hypothetical protein 1810539 1810964
NMB1728 biopolymer transport protein ExbD 1812088 1811657
NMB1729 biopolymer transport protein ExbB 1812753 1812094
NMB1730 TonB protein 1813661 1812822
NMB1731 conserved hypothetical protein 1813916 1814551
NMB1732 transporter, putative 1815806 1815009

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NMB1733 hypothetical protein 1816445 1815945
NMB1734 glutaredoxin 1817423 1816785
NMB1735 GTP pyrophosphokinase 1817566 1819776
NMB1736 transposase, putative FRAMESHIFT 1820048 1820856
NMB1737 secretion protein, putative 1822426 1821026
NMB1738 secretion protein, putative 1823922 1822498
NMB1739 hypothetical protein 1824158 1824508
NMB1740 hypothetical protein 1824635 1825042
NMB1741 conserved hypothetical protein FRAMESHIFT 1825116 1826455
NMB1742 hypothetical protein 1826503 1826790
NMB1743 hypothetical protein 1826798 1826992
NMB1744 hypothetical protein 1827003 1827284
NMB1745 hypothetical protein 1827294 1827569
NMB1746 hypothetical protein 1827700 1827987
NMB1747 tspB protein, putative 1828031 1829533
NMB1748 conserved hypothetical protein 1829537 1829824
NMB1749 conserved hypothetical protein 1829837 1830919
NMB1750 pilin gene inverting protein PivNM-2 1831548 1832495
NMB1751 transposase, degenerate 1833264 1832887
NMB1752 conserved hypothetical protein FRAMESHIFT 1833772 1833299
NMB1753 VapD-related protein 1834647 1835081
NMB1754 cryptic plasmid protein A-related protein 1835182 1835084
NMB1755 hypothetical protein 1835328 1835669
NMB1756 hypothetical protein 1835980 1836171
NMB1757 hypothetical protein 1836529 1836756
NMB1758 hypothetical protein 1837008 1837217
NMB1759 conserved hypothetical protein 1837403 1838764
NMB1760 conserved hypothetical protein 1839128 1839631
NMB1761 conserved hypothetical protein 1839797 1841047
NMB1762 hemolysin activation protein HecB, putative 1843162 1841378
NMB1763 toxin-activating protein, putative 1843675 1843220
NMB1764 hypothetical protein 1844155 1843844
NMB1765 hypothetical protein 1844466 1844170
NMB1766 hypothetical protein 1845460 1844450
NMB1767 hypothetical protein 1845945 1845532
NMB1768 hemagglutinin/hemolysin-related protein 1853493 1845952
NMB1769 IS1016 family transposase, putative truncation 1853631 1853822
NMB1770 transposase, IS30 family 1854072 1855034
NMB1771 hypothetical protein 1855539 1855108
NMB1772 hypothetical protein 1857374 1855539
NMB1773 hypothetical protein 1857783 1857412
NMB1774 hypothetical protein 1858438 1858064
NMB1775 hypothetical protein 1860252 1858450
NMB1776 hypothetical protein 1860353 1860252
NMB1777 hypothetical protein 1861364 1861122
NMB1778 hypothetical protein 1861489 1861388
NMB1779 hemagglutinin/hemolysin-related protein 1867499 1861515
NMB1780 hemolysin activation protein HecB, putative 1869350 1867611
NMB1781 hypothetical protein 1869919 1869752
NMB1782 hypothetical protein 1870236 1869937
NMB1783 secretion protein, putative FRAMESHIFT 1871826 1870605
NMB1784 hypothetical protein 1872240 1871890
NMB1785 hypothetical protein 1872472 1872236
NMB1786 hypothetical protein 1873623 1872472
NMB1787 N-acetyl-gamma-glutamyl-phosphate reductase 1874156 1875196
NMB1788 ATP-dependent DNA helicase RecG 1878304 1876265
NMB1789 protein-export protein SecB 1878833 1878393
NMB1790 glutaredoxin 3 1879111 1878857
NMB1791 cytoplasmic axial filament protein FRAMESHIFT 1879236 1880813
NMB1792 sensor histidine kinase 1881795 1880854
NMB1793 response regulator, putative FRAMESHIFT 1882272 1881854
NMB1794 citrate transporter 1883808 1882498
NMB1795 hypothetical protein 1884071 1883916
NMB1796 conserved hypothetical protein 1884950 1884381

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NMB1797 penicillin-binding protein 3 1885109 1886515
 NMB1798 IS1016 family transposase, putative FRAMESHIFT 1887236 1886597
 NMB1799 S-adenosylmethionine synthetase 1888654 1887488
 NMB1800 hypothetical protein 1888703 1888903
 NMB1801 HtrB/MsbB family protein 1889000 1889893
 NMB1802 O-sialoglycoprotein endopeptidase 1891004 1889943
 NMB1803 cytochrome c-type biogenesis protein, putative 1892308 1891124
 NMB1804 cytochrome c-type biogenesis protein, putative 1894316 1892304
 NMB1805 cytochrome c4 1895153 1894533
 NMB1806 conserved hypothetical protein 1895353 1895985
 NMB1807 penicillin-binding protein 1 1898505 1896112
 NMB1808 pilM protein 1898657 1899769
 NMB1809 pilN protein FRAMESHIFT 1899775 1900371
 NMB1810 pilO protein 1900375 1901019
 NMB1811 pilP protein 1901040 1901582
 NMB1812 pilQ protein FRAMESHIFT 1901604 1903908
 NMB1813 shikimate kinase 1904813 1905322
 NMB1814 3-dehydroquinate synthase 1905405 1906481
 NMB1815 conserved hypothetical protein 1907451 1908290
 NMB1816 conserved hypothetical protein 1908323 1908784
 NMB1817 riboflavin-specific deaminase 1908819 1909925
 NMB1818 lipopolysaccharide biosynthesis protein, putative 1910123 1911541
 NMB1819 hypothetical protein 1911541 1911693
 NMB1820 pilin glycosylation protein PglB 1911712 1912950
 NMB1821 pilin glycosylation protein PglC 1913086 1914258
 NMB1822 pilin glycosylation protein PglD 1914309 1916216
 NMB1823 valine--pyruvate aminotransferase 1916275 1917564
 NMB1824 conserved hypothetical protein 1918455 1917622
 NMB1825 hypothetical protein 1919103 1918903
 NMB1826 conserved hypothetical protein 1919452 1919084
 NMB1827 DNA polymerase III, alpha subunit 1919852 1923283
 NMB1828 conserved hypothetical protein 1924652 1923723
 NMB1829 TonB-dependent receptor 1926848 1924725
 NMB1830 phosphoglycolate phosphatase, putative 1926996 1927652
 NMB1831 lytB protein 1928711 1927746
 NMB1832 lipoprotein signal peptidase 1929267 1928743
 NMB1833 isoleucyl-tRNA synthetase 1933332 1930546
 NMB1834 riboflavin kinase/FMN adenylyltransferase 1934394 1933477
 NMB1835 tyrosyl-tRNA synthetase 1936217 1934925
 NMB1836 lipopolysaccharide biosynthesis protein WbpC, putative 1938151
 1936283
 NMB1837 hypothetical protein 1938466 1938215
 NMB1838 GTP-binding protein, putative 1939615 1938527
 NMB1839 formate--tetrahydrofolate ligase 1941406 1939733
 NMB1840 conserved hypothetical protein 1941581 1942009
 NMB1841 mannose-1-phosphate guanylyltransferase-related protein 1942741
 1942049
 NMB1842 4-hydroxyphenylacetate 3-hydroxylase, small subunit, putative
 1943257 1942760
 NMB1843 transcriptional regulator, MarR family 1943812 1943375
 NMB1844 hypothetical protein 1943938 1943819
 NMB1845 thioredoxin 1944662 1944156
 NMB1846 Mrp/NBP35 family protein 1945032 1946108
 NMB1847 pilC1 protein FRAMESHIFT 1947287 1950374
 NMB1848 hypothetical protein 1952279 1951938
 NMB1849 carbamoyl-phosphate synthase, small subunit 1952589 1953719
 NMB1850 hypothetical protein 1954091 1954363
 NMB1851 hypothetical protein 1954440 1954697
 NMB1852 conserved hypothetical protein 1954697 1955083
 NMB1853 hypothetical protein 1955422 1955691
 NMB1854 hypothetical protein 1955768 1956406
 NMB1855 carbamoyl-phosphate synthase, large subunit 1956438 1959650
 NMB1856 transcriptional regulator, LysR family 1960777 1959881
 NMB1857 modulator of drug activity B 1961016 1961591

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NMB1858 hypothetical protein 1961977 1961594
NMB1859 S-adenosylmethionine:tRNA ribosyltransferase-isomerase 1963108
1962071
NMB1860 acetyl-CoA carboxylase, biotin carboxyl carrier protein 1963464
1963916
NMB1861 acetyl-CoA carboxylase, biotin carboxylase 1964031 1965389
NMB1862 ribosomal protein L11 methyltransferase 1965653 1966537
NMB1863 oligoribonuclease 1966558 1967118
NMB1864 glutamate-1-semialdehyde 2,1-aminomutase 1968808 1967528
NMB1865 hypothetical protein 1968821 1969036
NMB1866 conserved hypothetical protein 1969593 1970918
NMB1867 1-deoxyxylulose-5-phosphate synthase 1972919 1971009
NMB1868 integrase/recombinase XerC 1973909 1973007
NMB1869 fructose-bisphosphate aldolase 1974093 1975154
NMB1870 hypothetical protein 1975177 1976136
NMB1871 conserved hypothetical protein 1976286 1976960
NMB1872 ribosomal-protein-alanine acetyltransferase, putative 1976960
1977397
NMB1873 DNA polymerase, bacteriophage-type, putative 1977394 1978128
NMB1874 orotate phosphoribosyltransferase 1978193 1978831
NMB1875 hypothetical protein 1978908 1979339
NMB1876 N-acetylglutamate synthase 1979339 1980646
NMB1877 prolyl oligopeptidase family protein 1980850 1982862
NMB1878 transcriptional regulator, AraC family 1983567 1982983
NMB1879 hypothetical protein 1983936 1983628
NMB1880 ABC transporter, periplasmic solute-binding protein, putative
1984172 1985134
NMB1881 conserved hypothetical protein 1985694 1986014
NMB1882 TonB-dependent receptor 1986131 1988305
NMB1883 hypothetical protein 1988727 1988440
NMB1884 conserved hypothetical protein 1989047 1988727
NMB1885 protein-L-isoaspartate O-methyltransferase 1989783 1989130
NMB1886 conserved hypothetical protein 1990389 1989889
NMB1887 triosephosphate isomerase 1990568 1991338
NMB1888 protein-export membrane protein SecE 1991348 1991695
NMB1889 hypothetical protein 1992486 1992575
NMB1890 conserved hypothetical protein 1992709 1993074
NMB1891 helix-turn-helix family protein 1993074 1993382
NMB1892 hypothetical protein 1993495 1993704
NMB1893 conserved hypothetical protein FRAMESHIFT 1994615 1993771
NMB1894 leucyl-tRNA synthetase, truncation 1994851 1994723
NMB1895 DNA adenine methylase, truncation 1994987 1994847
NMB1896 type II restriction enzyme DpnI 1995774 1994974
NMB1897 leucyl-tRNA synthetase 1998538 1995911
NMB1898 lipoprotein 1998808 1999320
NMB1899 hypothetical protein 1999330 1999770
NMB1900 polyphosphate kinase 1999849 2001996
NMB1901 IS1016C2 transposase, degenerate 2002232 2002770
NMB1902 DNA polymerase III, beta subunit 2004113 2003013
NMB1903 chromosomal replication initiator protein DnaA 2005904 2004351
NMB1904 ribosomal protein L34 2006196 2006327
NMB1905 ribonuclease P protein component 2006333 2006695
NMB1906 conserved hypothetical protein 2006763 2006981
NMB1907 60 kd inner-membrane protein 2007156 2008790
NMB1908 conserved hypothetical protein 2009599 2008877
NMB1909 Maf/YceF/YhdE family protein 2010236 2009649
NMB1910 conserved hypothetical protein 2010384 2010884
NMB1911 50S ribosomal protein L32 2010921 2011097
NMB1912 conserved hypothetical protein 2011275 2011799
NMB1913 fatty acid/phospholipid synthesis protein 2011891 2012943
NMB1914 hypothetical protein 2013082 2013330
NMB1915 hypothetical protein 2013360 2013746
NMB1916 3-oxoacyl-(acyl-carrier-protein) synthase III 2013931 2014890
NMB1917 conserved hypothetical protein 2014940 2015344

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NMB1918 malonyl CoA-acyl carrier protein transacylase 2015441 2016364
 NMB1919 ABC transporter, ATP-binding protein 2016505 2018367
 NMB1920 GMP synthase 2018470 2020032
 NMB1921 3-oxoacyl-(acyl-carrier-protein) reductase 2020097 2020840
 NMB1922 IS1106 transposase, degenerate 2021273 2021118
 NMB1923 conserved hypothetical protein 2021377 2021757
 NMB1924 inositol monophosphatase family protein 2022673 2021981
 NMB1925 conserved hypothetical protein 2022876 2023598
 NMB1926 lacto-N-neotetraose biosynthesis glycosyl transferase LgtE 2025680
 2024841
 NMB1927 lacto-N-neotetraose biosynthesis glycosyl transferase-related
 protein 2025817 2025725
 NMB1928 lacto-N-neotetraose biosynthesis glycosyl transferase LgtB 2026656
 2025832
 NMB1929 lacto-N-neotetraose biosynthesis glycosyl transferase LgtA 2027747
 2026701
 NMB1930 glycyl-tRNA synthetase, beta chain 2029827 2027767
 NMB1931 hypothetical protein 2030256 2029912
 NMB1932 glycyl-tRNA synthetase, alpha chain 2031238 2030336
 NMB1933 ATP synthase F1, epsilon subunit 2032065 2031646
 NMB1934 ATP synthase F1, beta subunit 2033473 2032079
 NMB1935 ATP synthase F1, gamma subunit 2034386 2033514
 NMB1936 ATP synthase F1, alpha subunit 2035958 2034414
 NMB1937 ATP synthase F1, delta subunit 2036502 2035972
 NMB1938 ATP synthase F0, B subunit 2036977 2036510
 NMB1939 ATP synthase F0, C subunit 2037284 2037051
 NMB1940 ATP synthase F0, A subunit 2038207 2037344
 NMB1941 hypothetical protein 2038550 2038200
 NMB1942 hypothetical protein 2038997 2038707
 NMB1943 hypothetical protein 2039340 2039170
 NMB1944 ParB family protein 2040252 2039395
 NMB1945 3-octaprenyl-4-hydroxybenzoate carboxy-lyase 2040407 2040976
 NMB1946 outer membrane lipoprotein 2041904 2041044
 NMB1947 ABC transporter, permease protein 2042749 2042066
 NMB1948 ABC transporter, ATP-binding protein 2043488 2042754
 NMB1949 soluble lytic murein transglycosylase, putative 2044018 2045865
 NMB1950 30S ribosomal protein S21 2046157 2046366
 NMB1951 conserved hypothetical protein 2046405 2046944
 NMB1952 stringent starvation protein B 2047538 2047149
 NMB1953 stringent starvation protein A 2048215 2047613
 NMB1954 hypothetical protein 2050146 2048488
 NMB1955 cadmium resistance protein 2050933 2050310
 NMB1956 50S ribosomal protein L31 2051451 2051239
 NMB1957 acetyltransferase-related protein FRAMESHIFT 2051688 2052197
 NMB1958 thioredoxin, putative 2052770 2052273
 NMB1959 conserved hypothetical protein 2053150 2052770
 NMB1960 hypothetical protein 2053632 2053153
 NMB1961 VacJ-related protein 2054464 2053640
 NMB1962 hypothetical protein 2054739 2054464
 NMB1963 conserved hypothetical protein 2055380 2054793
 NMB1964 conserved hypothetical protein 2055911 2055420
 NMB1965 conserved hypothetical protein 2056738 2055965
 NMB1966 ABC transporter, ATP-binding protein 2057586 2056789
 NMB1967 transcriptional regulator, AraC family 2057759 2058673
 NMB1968 aldehyde dehydrogenase A 2058936 2060375
 NMB1969 serotype-1-specific antigen, putative 2061412 2064657
 NMB1970 para-aminobenzoate synthetase component I/4-amino-4-
 deoxychorismate lyase, putative 2065692 2067470
 NMB1971 conserved hypothetical protein 2069049 2067535
 NMB1972 chaperonin, 60 kDa 2071379 2069748
 NMB1973 chaperonin, 10 kDa 2071762 2071475
 NMB1974 IS1016C2 transposase, degenerate 2071990 2072639
 NMB1975 sodium- and chloride-dependent transporter 2072855 2074387
 NMB1976 diaminopimelate decarboxylase 2075759 2074518

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NMB1977 hypothetical protein 2075940 2075773
 NMB1978 cyaY protein 2076011 2076331
 NMB1979 conserved hypothetical protein 2076361 2077374
 NMB1980 conserved hypothetical protein 2077403 2077819
 NMB1981 conserved hypothetical protein 2077844 2078347
 NMB1982 DNA polymerase I 2078496 2081309
 NMB1983 hypothetical protein 2082658 2083326
 NMB1984 IS1106 transposase FRAMESHIFT 2083391 2084499
 NMB1985 adhesion and penetration protein 2089191 2084821
 NMB1986 hypothetical protein 2089756 2089328
 NMB1987 thiophene and furan oxidation protein ThdF 2090041 2091384
 NMB1988 iron-regulated outer membrane protein FrpB 2092611 2094752
 NMB1989 iron(III) ABC transporter, periplasmic binding protein 2095472
 2096434
 NMB1990 iron(III) ABC transporter, permease protein 2096601 2097566
 NMB1991 iron(III) ABC transporter, permease protein 2097559 2098530
 NMB1992 hypothetical protein 2098577 2099200
 NMB1993 iron(III) ABC transporter, ATP-binding protein 2099286 2100041
 NMB1994 adhesin/invasin, putative 2100342 2101433
 NMB1995 nitrogen regulatory protein P-II, FRAMESHIFT 2101839 2101423
 NMB1996 phosphoribosylformylglycinamide synthase 2101990 2105949
 NMB1997 hydroxyacylglutathione hydrolase 2106047 2106802
 NMB1998 serine-type peptidase 2107119 2111411
 NMB1999 magnesium transporter 2111646 2113097
 NMB2000 conserved hypothetical protein 2114094 2113189
 NMB2001 conserved hypothetical protein 2114339 2115091
 NMB2002 hypothetical protein 2115113 2115328
 NMB2003 conserved hypothetical protein 2115476 2115820
 NMB2004 conserved hypothetical protein 2115820 2116509
 NMB2005 glutamate N-acetyltransferase/amino-acid acetyltransferase 2116579
 2117796
 NMB2006 chloride channel protein-related protein 2117859 2119265
 NMB2007 ATP-dependent RNA helicase HrpA, truncation 2119458 2120846
 NMB2008 ABC transporter, ATP-binding protein-related protein 2120993
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 NMB2009 ATP-dependent RNA helicase HrpA, degenerate 2122680 2122859
 NMB2010 YhbX/YhjW/YijP/YjdB family protein 2123074 2124648
 NMB2011 ATP-dependent RNA helicase HrpA, truncation 2124717 2128133
 NMB2012 transcriptional regulator, HTH 3 family 2129260 2128172
 NMB2013 hypothetical protein 2129920 2129279
 NMB2014 hypothetical protein 2130249 2130004
 NMB2015 hypothetical protein 2130614 2130880
 NMB2016 type IV pilin-related protein 2131493 2131047
 NMB2017 ComeA-related protein 2132027 2131584
 NMB2018 conserved hypothetical protein 2138411 2137752
 NMB2019 lipopolysaccharide core biosynthesis protein KdtB 2138949 2138440
 NMB2020 conserved hypothetical protein 2139756 2139076
 NMB2021 conserved hypothetical protein 2140179 2139916
 NMB2022 conserved hypothetical protein 2140722 2140255
 NMB2023 conserved hypothetical protein 2141162 2140779
 NMB2024 conserved hypothetical protein 2141826 2141224
 NMB2025 conserved hypothetical protein 2142422 2141826
 NMB2026 ABC transporter, permease protein 2144046 2142454
 NMB2027 gluconate permease 2144385 2145767
 NMB2028 thermoresistant gluconokinase 2145790 2146305
 NMB2029 homoserine kinase FRAMESHIFT 2147564 2146650
 NMB2030 3-demethylubiquinone-9 3-methyltransferase 2148329 2147604
 NMB2031 tryptophan transporter 2148481 2149719
 NMB2032 lipopolysaccharide glycosyl transferase, FRAMESHIFT 2149872
 2150922
 NMB2033 histidinol-phosphatase, putative 2151173 2151733
 NMB2034 1-acyl-sn-glycerol-3-phosphate acyltransferase, putative 2151765
 2152505
 NMB2035 conserved hypothetical protein 2152505 2153194

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NMB2036 tRNA pseudouridine synthase A 2154495 2155390
NMB2037 hypothetical protein 2155415 2155651
NMB2038 PemK-related protein 2155642 2155962
NMB2039 major outer membrane protein PIB 2157487 2158479
NMB2040 thiamine biosynthesis protein ThiC 2161479 2159581
NMB2041 thiamin pyrophosphokinase-related protein 2162093 2162965
NMB2042 spermidine/putrescine ABC transporter, ATP-binding protein 2162977
2163912
NMB2043 IS1106 transposase, putative POINT MUTATION 2165702 2164734
NMB2044 phosphoenolpyruvate-protein phosphotransferase 2168278 2166506
NMB2045 phosphocarrier protein HPr 2168547 2168281
NMB2046 PTS system, IIAB component 2169074 2168619
NMB2047 hypoxanthine-guanine phosphoribosyltransferase, putative 2169697
2169137
NMB2048 DNA ligase 2170590 2169769
NMB2049 glyoxalase II family protein 2170682 2171311
NMB2050 conserved hypothetical protein 2173305 2171524
NMB2051 ubiquinol--cytochrome c reductase, cytochrome c1 2174444 2173647
NMB2052 ubiquinol--cytochrome c reductase, cytochrome b 2175793 2174447
NMB2053 ubiquinol--cytochrome c reductase, iron-sulfur subunit 2176393
2175815
NMB2054 conserved hypothetical protein 2177265 2176519
NMB2055 transcriptional regulator, LysR family 2177396 2178322
NMB2056 30S ribosomal protein S9 2178972 2178583
NMB2057 50S ribosomal protein L13 2179413 2178985
NMB2058 conserved hypothetical protein 2180081 2179779
NMB2059 hypothetical protein 2180421 2180095
NMB2060 glycerol-3-phosphate dehydrogenase (NAD+) 2181465 2180479
NMB2061 phosphoenolpyruvate carboxylase 2184290 2181591
NMB2062 thiF protein 2184460 2185227
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NMB2064 conserved hypothetical protein 2187407 2186022
NMB2065 hemK protein FRAMESHIFT 2188764 2187496
NMB2066 tldD protein 2190271 2188832
NMB2067 conserved hypothetical protein 2190661 2191881
NMB2068 D-amino acid oxidase flavoprotein, putative 2191881 2192978
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NMB2076 aut protein 2199160 2198657
NMB2077 methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate
cyclohydrolase FRAMESHIFT 2199800 2200650
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NMB2092 hypothetical protein 2212437 2213066
NMB2093 methionine aminopeptidase 2213109 2213885
NMB2094 hypothetical protein 2214043 2214339
NMB2095 adhesin complex protein, putative 2214580 2214951

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NMB2096 malate:quinone oxidoreductase 2216608 2215145
NMB2097 hypothetical protein 2216749 2216663
NMB2098 conserved hypothetical protein 2217735 2217148
NMB2099 conserved hypothetical protein 2218377 2217799
NMB2100 hypothetical protein 2218455 2218685
NMB2101 30S ribosomal protein S2 2218861 2219586
NMB2102 elongation factor TS (EF-TS) 2219718 2220569
NMB2103 uridylate kinase 2220789 2221505
NMB2104 mafA protein FRAMESHIFT 2221692 2222652
NMB2105 mafB protein 2222695 2224143
NMB2106 hypothetical protein 2224143 2224496
NMB2107 MafB-related protein 2224527 2225288
NMB2108 hypothetical protein 2225301 2225504
NMB2109 hypothetical protein 2225639 2225887
NMB2110 hypothetical protein 2225887 2226255
NMB2111 MafB-related protein 2226268 2227110
NMB2112 hypothetical protein 2227306 2227572
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NMB2114 MafB-related protein 2227948 2228583
NMB2115 hypothetical protein 2228589 2228930
NMB2116 hypothetical protein 2228971 2229312
NMB2117 MafB-related protein, degenerate 2229645 2230340
NMB2118 hypothetical protein 2230340 2230654
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NMB2121 hypothetical protein 2232031 2232372
NMB2122 MafB-related protein 2232409 2232510
NMB2123 hypothetical protein 2232518 2232871
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NMB2125 hypothetical protein 2233047 2233418
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NMB2127 protease, putative 2235364 2234381
NMB2128 CinA-related protein 2236204 2235407
NMB2129 argininosuccinate synthase 2236517 2237857
NMB2130 hypothetical protein 2237908 2238147
NMB2131 hypothetical protein 2238143 2238355
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NMB2134 conserved hypothetical protein 2241857 2243761
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NMB2137 hypothetical protein 2251451 2251660
NMB2138 peptide chain release factor 2 2252924 2251824
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NMB2146 hypothetical protein 2257703 2257810
NMB2147 hypothetical protein 2257842 2258261
NMB2148 transposase, IS30 family 2258738 2259700
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NMB2152 hypothetical protein 2262502 2262816
NMB2153 conserved hypothetical protein 2263482 2262874
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NMB2155 electron transfer flavoprotein, beta subunit 2265240 2264494
NMB2156 heptosyltransferase I 2266435 2265470
NMB2157 pyrazinamidase/nicotinamidase PncA, putative 2267107 2266475
NMB2158 conserved hypothetical protein 2267221 2267898
NMB2159 glyceraldehyde 3-phosphate dehydrogenase 2269163 2268162

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NMB2160 DNA mismatch repair protein MutS 2269607 2272198
NMB0505 hypothetical protein 533467 533186
NMB1123 hypothetical protein 1135584 1135390
NMB1124 hypothetical protein 1136271 1135627
NMB1125 hypothetical protein 1136639 1136271
NMB1126 hypothetical protein 1137317 1136649
NMB1127 oxidoreductase, short chain dehydrogenase/reductase family 1138201
1137485
NMB1129 hypothetical protein 1139833 1139630
NMB1130 phytoene synthase, putative 1140867 1139998
NMB1133 conserved hypothetical protein / ankyrin-related protein 1144428
1143670
NMB1134 ferredoxin, 2Fe-2S type 1144824 1144486
NMB1135 hypothetical protein 1145242 1145102
NMB1137 conserved hypothetical protein 1146211 1146017
NMB1138 conserved hypothetical protein 1146683 1146285
NMB1141 RNA methyltransferase, TrmH family 1150088 1149480
NMB1142 hypothetical protein 1150375 1150142
NMB1143 hypothetical protein 1150909 1150547
NMB1144 hypothetical protein 1151226 1150924, lipoprotein
NMB1147 hypothetical protein 1154639 1154007, homology to plasmid proteins
Y4SH RISHN and PXO2 BACAN
NMB1149 hypothetical protein 1155016 1154876
NMB1151 sulfite reductase hemoprotein, beta-component 1159086 1157320
NMB1152 sulfite reductase (NADPH) flavoprotein, alpha component 1160927
1159116
NMB1154 sulfate adenylyltransferase, subunit 2 1163172 1162252
NMB1156 siroheme synthase 1165412 1163964
NMB1157 hypothetical protein 1165696 1165541
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1178931
NMB1178 mesJ protein FRAMESHIFT 1181265 1179984
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diaminopimelate ligase 1184700 1183327
NMB1184 biotin synthetase 1185959 1184910
NMB1186 hypothetical protein 1186881 1186729
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NMB1191 sulfate adenylyltransferase, subunit 1 1194246 1192963
NMB1193 phosphoadenosine phosphosulfate reductase 1195986 1195249
NMB1196 nickel-dependent hydrogenase, b-type cytochrome subunit 1198401
1197748

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/68 C12N15/11 C07K14/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 17805 A (RAYMOND NIGEL ;QUINN FREDERICK D (US); US HEALTH (US); RIBOT EFRAI) 30 April 1998 (1998-04-30) the whole document	1-4, 7-14, 18-24
X	EP 0 467 714 A (MERCK & CO INC) 22 January 1992 (1992-01-22) claims; example 3 -/--	1-4, 7-14, 18-24



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

10 October 2000

Date of mailing of the international search report

19.10.00

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Luzzatto, E

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>FLEISCHMANN R D ET AL: "WHOLE-GENOME RANDOM SEQUENCING AND ASSEMBLY OF HAEMOPHILUS INFLUENZAE RD" SCIENCE,US,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 269, no. 5223, 28 July 1995 (1995-07-28), pages 496-498,507-51, XP000517090 ISSN: 0036-8075 the whole document</p>	<p>1-4, 7-14, 16-24</p>
T	<p>TETTELIN H ET AL: "Complete genome sequence of Neisseria meningitidis serogroup B strain MC58 'see comments!.' SCIENCE, (2000 MAR 10) 287 (5459) 1809-15., XP000914963 page 963</p>	
T	<p>PIZZA M ET AL: "Identification of vaccine candidates against serogroup B meningococcus by whole- genome sequencing 'see comments!.' SCIENCE, (2000 MAR 10) 287 (5459) 1816-20., XP000914964 the whole document</p>	
T	<p>PARKHILL J ET AL: "Complete DNA sequence of a serogroup A strain of Neisseria meningitidis Z2491 'see comments!.' NATURE, (2000 MAR 30) 404 (6777) 502-6., XP000918875 the whole document</p>	

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: **16,17 (partly)**
because they relate to subject matter not required to be searched by this Authority, namely:
Rule 39.1(v) PCT - Presentation of information (insofar as related to computer databases)
2. ☒ Claims Nos.: **5,6,15 (completely), 1-4, 7-14, 16-24 (partly)**
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of Box I.2

Claims Nos.: 5,6,15 (completely), 1-4, 7-14, 16-24 (partly)

1) Claims 5 and 6 (and thus 15 which refers to claim 6 and whose reference to claims 7 and 8 is wrong) lack any essential technical feature which could allow a meaningful search to be carried out. They have thus not been searched. For the same reason claims 18-24 have not been searched insofar as referring to any of claims 5, 6 and 15.

2) Claims 1-4, 7-14, 16-24 have only been searched insofar as related to the full sequence SEQ ID 1 in view of the absence of any indication in the claims as to searcheable SEQ IDs corresponding to the "NMB open reading frames". SEQ ID 1 as such is not searchable by means of similarity algorithms since it is too long: the search with respect thereto has thus been carried out based on keywords.

3) A further reason for not searching claims 1-4 insofar as related to "NMB open reading frames" is that claim 1 is unclear (Art. 6 PCT). It relates to a method for searching open reading frames "within one or more...NMB open reading frames", which is however technically meaningless.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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